

APOLIPOPROTEIN C3 (APOC3) IRNA COMPOSITIONS AND METHODS OF USE THEREOF

Related Applications

5 The present application claims the benefit of priority to U.S. Provisional Application No. 63/144,516, filed on February 2, 2021, and U.S. Provisional Application No. 62/977,875, filed on February 18, 2020. The entire contents of each of the foregoing applications are incorporated herein by reference.

Sequence Listing

10 The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on February 10, 2021, is named 121301_10420_SL.TXT and is 249,455 bytes in size.

Background of the Invention

15 Apolipoprotein C3 (APOC3) is a very low density lipoprotein (VLDL) and an important regulator of lipoprotein metabolism. In humans, APOC3 is encoded by the APOC3 gene that is located in a gene cluster together with the APOA1 and APOA4 genes on the long arm of chromosome 11. APOC3 is expressed in the liver and, to a lesser extent, in the intestines, as a small 99-amino acid protein.

20 Following removal of the 20-amino-acid signal peptide in the endoplasmic reticulum, a mature ApoC3 protein of 79 amino acids is formed, which may be present as a non-glycosylated or a glycosylated isoform.

 The primary role of APOC3 is as a regulator of lipolysis through non-competitive inhibition of endothelial bound lipoprotein lipase (LPL). LPL hydrolyses triacylglycerols in triacylglycerol

25 (triglyceride)-rich lipoproteins (TRLs), releasing fatty acids into the plasma and transforming large triacylglycerol-rich particles into smaller triacylglycerol-depleted remnant lipoproteins. Individuals lacking APOC3 have low TRL levels, coupled with highly efficient lipolysis of triacylglycerols. Furthermore, mice in which the APOC3 gene has been genetically deleted were shown to have low plasma triacylglycerol levels and efficient TRL catabolism. APOC3 also inhibits hepatic lipase (HL), a

30 lipolytic enzyme with triacylglycerol lipase and phospholipase A1 activity that is synthesized in the liver. The inhibitory effect of APOC3 on HL further reduces the lipolysis and uptake of TRL remnants by the liver. APOC3 has also been shown to stimulate synthesis of very low density lipoproteins (VLDLs). It is believed that the underlying mechanisms associated with this effect of APOC3 may relate to the inhibition of proteasome mediated degradation of APOB, resulting in increased APOB synthesis and secretion, and

35 increased synthesis of VLDL triacylglycerols. APOC3 may, therefore, play a key role in regulating VLDL output by the liver.

 Cellular studies report that APOC3 may interfere with TRL and remnant binding to hepatic lipoprotein receptors. APOC3 can abolish APOB- and ApoE-mediated binding of lipoproteins to low density lipoprotein receptor (LDLR), either by masking or altering the conformation of APOB and APOE.

The binding of chylomicrons and VLDL particles to the lipolysis-stimulated receptor (LSR) is also significantly inhibited by APOC3.

An increase in APOC3 levels induces the development of hypertriglyceridemia, or high (hyper-) blood levels (-emia) of triglycerides. Elevated levels of triglycerides are associated with a variety of diseases, including cardiovascular disease, atherosclerosis, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, polycystic ovary syndrome, kidney disease, obesity, type 2 diabetes mellitus (insulin resistance), hypertension and skin lesions (xanthomas). Very high triglyceride levels also increase the risk of acute pancreatitis. Therefore, regulating APOC3 metabolism is an important therapeutic approach to managing hypertriglyceridemia and the associated diseases.

Accordingly, there is a need in the art for regulators of APOC3 expression for treating apolipoprotein C3-associated disorders, such as hypertriglyceridemia.

Summary of the Invention

The present invention provides iRNA compositions which affect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a gene encoding apolipoprotein C3 (APOC3). The apolipoprotein C3 (APOC3) may be within a cell, *e.g.*, a cell within a subject, such as a human subject.

In an aspect, the invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of apolipoprotein C3 in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of SEQ ID NO:1 and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 1, 2, or 3 nucleotides from the nucleotide sequence of SEQ ID NO:2. In one embodiment, the dsRNA agent comprises at least one thermally destabilizing nucleotide modification, *e.g.*, an abasic modification; a mismatch with the opposing nucleotide in the duplex; and destabilizing sugar modification, a 2'-deoxy modification, an acyclic nucleotide, an unlocked nucleic acids (UNA), or a glycerol nucleic acid (GNA), *e.g.*, the antisense strand comprises at least one thermally destabilizing nucleotide modification.

In another aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) for inhibiting expression of apolipoprotein C3 in a cell, wherein said dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises a region of complementarity to an mRNA encoding apolipoprotein C3, and wherein the region of complementarity comprises at least 15 contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from any one of the antisense nucleotide sequences in any one of Tables 2-5, 14, and 15.

In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) for inhibiting expression of apolipoprotein C3 in a cell, wherein said dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from any one of the

nucleotide sequence of nucleotides; 232-254; 233-255; 238-260; 239-261; 242-264; 243-265; 244-266; 264-286; 268-290; 426-448; 431-453; 432-454; 433-455; 435-457; 436-458; 499-521; 500-522; 503-525; 504-526; 507-529; 510-532; or 511-533 of the nucleotide sequence of SEQ ID NO:1, and the antisense strand comprises at least 19 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

In one embodiment, the antisense strand comprises at least 15 contiguous nucleotides differing by not more than 0, 1, 2, or 3 nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-959917.1; AD-959918.1; AD-960096.1; AD-960064.1; AD-959914.1; AD-959941.1; AD-960031.1; AD-960063.1; AD-960293.1; AD-960288.1; AD-960445.1; AD-960292.1; AD-960475.1; AD-960442.1; AD-960470.1; AD-960436.1; AD-960446.1; AD-960474.1; AD-960294.1; AD-960471.1; AD-960314.1; AD-960443.1; AD-960282.1; AD-960283.1; AD-80794.7; AD-960478.1; AD-960481.1; and AD-960482.1.

In another aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) for inhibiting expression of apolipoprotein C3 in a cell, wherein the dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from any one of the nucleotide sequence of nucleotides 235-257; 238-260; 242-264; 243-265; 244-266; 426-448; 430-450; 431-453; 432-454; 433-455; 435-457; 436-458; 499-521; 503-525; and 504-526 of the nucleotide sequence of SEQ ID NO:1, and the antisense strand comprises at least 19 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

In one embodiment, the antisense strand comprises at least 15 contiguous nucleotides differing by not more than 0, 1, 2, or 3 nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-959917.1; AD-960064.1; AD-960031.1; AD-960063.1; AD-960293.1; AD-960288.1; AD-960445.1; AD-960292.1; AD-960475.1; AD-960442.1; AD-960470.1; AD-960436.1; AD-960446.1; AD-960474.1; AD-960294.1; AD-960443.1; AD-80794.7; and AD-959910.1.

In another aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) for inhibiting expression of apolipoprotein C3 in a cell, wherein the dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from any one of the nucleotide sequence of nucleotides 232-254; 239-261; 242-264; 244-266; 258-280; 264-286; 268-290; 429-451; 430-450; 430-452; 433-455; 434-456; 435-457; 500-522; 503-525; 507-529; and 510-532 of the nucleotide sequence of SEQ ID NO:1, and the antisense strand comprises at least 19 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

In one embodiment, the antisense strand comprises at least 15 contiguous nucleotides differing by not more than 0, 1, 2, or 3 nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-80794.8; AD-959907.2; AD-959914.2; AD-959916.2; AD-959932.2; AD-960314.2; AD-959941.2; AD-960030.2; AD-960062.2;

AD-960064.2; AD-960065.2; AD-960066.2; AD-960294.2; AD-960471.2; AD-960474.2; AD-960478.2; and AD-960481.2.

In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) for inhibiting expression of apolipoprotein C3 in a cell, wherein the dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from any one of the nucleotide sequence of nucleotides 429-455 or 504-532 of the nucleotide sequence of SEQ ID NO:1, and the antisense strand comprises at least 19 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

In another aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) for inhibiting expression of apolipoprotein C3 in a cell, wherein the dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from any one of the nucleotide sequence of nucleotides 429-451; 430-452; 431-451; 432-452; 433-455; 434-452; 504-526; and 506-526 of the nucleotide sequence of SEQ ID NO:1, and the antisense strand comprises at least 19 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

In one embodiment, the antisense strand comprises at least 15 contiguous nucleotides differing by not more than 0, 1, 2, or 3 nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-960030; AD-960064; AD-1143243; AD-1143245; AD-1143247; AD-1143249; AD-1143256; AD-1143260; AD-1143278; AD-1143287; AD-1143295; AD-1143299; AD-1143302; and AD-1143305.

In one embodiment, the antisense strand comprises at least 15 contiguous nucleotides differing by not more than 0, 1, 2, or 3 nucleotides from the antisense strand nucleotide sequence of duplex AD-1143278 or AD-960064.

In one embodiment, the antisense strand comprises at least 15 contiguous nucleotides differing by not more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of nucleotides 429-456 of SEQ ID NO:1.

In one embodiment, the sense strand comprises at least 15 contiguous nucleotides differing by not more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of nucleotides 433-455 of SEQ ID NO:1.

In one embodiment, the sense strand comprises at least 15 contiguous nucleotides differing by not more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of nucleotides 434-452 of SEQ ID NO:1.

In one embodiment, the antisense strand comprises at least 15 contiguous nucleotides differing by not more than 0, 1, 2, or 3 nucleotides from the antisense strand nucleotide sequence of duplex AD-1143243.

In one embodiment, the dsRNA agent comprises at least one modified nucleotide.

In one embodiment, substantially all of the nucleotides of the sense strand; substantially all of the nucleotides of the antisense strand comprise a modification; or substantially all of the

nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand comprise a modification.

In one embodiment, all of the nucleotides of the sense strand comprise a modification; all of the nucleotides of the antisense strand comprise a modification; or all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a modification.

In one embodiment, at least one of the modified nucleotides is selected from the group consisting of a deoxy-nucleotide, a 3'-terminal deoxy-thymine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-O-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, 2'-hydroxyl-modified nucleotide, a 2'-methoxyethyl modified nucleotide, a 2'-O-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a phosphorothioate group, a nucleotide comprising a methylphosphonate group, a nucleotide comprising a 5'-phosphate, a nucleotide comprising a 5'-phosphate mimic, a thermally destabilizing nucleotide, a glycol modified nucleotide (GNA), and a 2-O-(N-methylacetamide) modified nucleotide; and combinations thereof.

In one embodiment, the modifications on the nucleotides are selected from the group consisting of LNA, HNA, CeNA, 2'-methoxyethyl, 2'-O-alkyl, 2'-O-allyl, 2'-C-allyl, 2'-fluoro, 2'-deoxy, 2'-hydroxyl, and glycol; and combinations thereof.

In one embodiment, at least one of the modified nucleotides is selected from the group consisting of a deoxy-nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a glycol modified nucleotide (GNA), *e.g.*, Ggn, Cgn, Tgn, or Agn, and, a vinyl-phosphonate nucleotide; and combinations thereof.

In another embodiment, at least one of the modifications on the nucleotides is a thermally destabilizing nucleotide modification.

In one embodiment, the thermally destabilizing nucleotide modification is selected from the group consisting of an abasic modification; a mismatch with the opposing nucleotide in the duplex; and destabilizing sugar modification, a 2'-deoxy modification, an acyclic nucleotide, an unlocked nucleic acids (UNA), and a glycerol nucleic acid (GNA).

The double stranded region may be 19-30 nucleotide pairs in length; 19-25 nucleotide pairs in length; 19-23 nucleotide pairs in length; 23-27 nucleotide pairs in length; or 21-23 nucleotide pairs in length.

In one embodiment, each strand is independently no more than 30 nucleotides in length.

In one embodiment, the sense strand is 21 nucleotides in length and the antisense strand is 23 nucleotides in length.

The region of complementarity may be at least 17 nucleotides in length; between 19 and 23 nucleotides in length; or 19 nucleotides in length.

In one embodiment, at least one strand comprises a 3' overhang of at least 1 nucleotide. In another embodiment, at least one strand comprises a 3' overhang of at least 2 nucleotides.

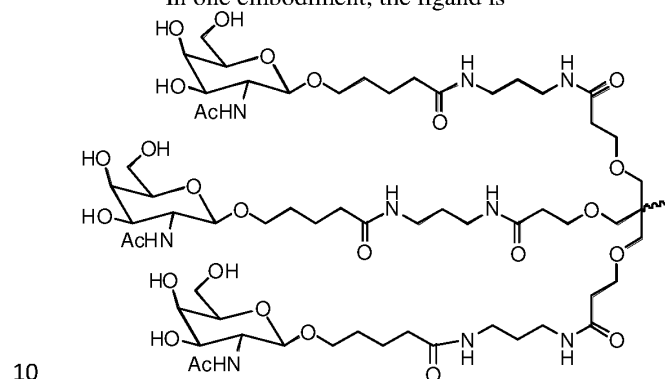
In one embodiment, the dsRNA agent further comprises a ligand.

In one embodiment, the ligand is conjugated to the 3' end of the sense strand of the dsRNA agent.

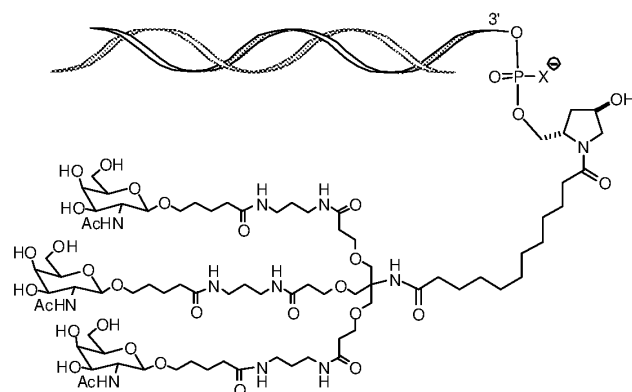
In one embodiment, the ligand is an N-acetylgalactosamine (GalNAc) derivative.

In one embodiment, the ligand is one or more GalNAc derivatives attached through a monovalent, bivalent, or trivalent branched linker.

In one embodiment, the ligand is



In one embodiment, the dsRNA agent is conjugated to the ligand as shown in the following schematic



and, wherein X is O or S.

15 In one embodiment, the X is O.

In one embodiment, the dsRNA agent further comprises at least one phosphorothioate or methylphosphonate internucleotide linkage.

In one embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at the 3'-terminus of one strand, *e.g.*, the antisense strand or the sense strand.

20 In another embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at the 5'-terminus of one strand, *e.g.*, the antisense strand or the sense strand.

In one embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at the both the 5'- and 3'-terminus of one strand. In one embodiment, the strand is the antisense strand.

In one embodiment, the base pair at the 1 position of the 5'-end of the antisense strand of the duplex is an AU base pair.

In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) for inhibiting expression of apolipoprotein C3 in a cell, wherein the dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of nucleotides 434-452 of the nucleotide sequence of SEQ ID NO:1, and the antisense strand comprises at least 19 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2, wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand comprise a modification selected from the group consisting of a 2'-O-methyl modification, a 2'-fluoro modification, and a deoxy-modification, wherein both the sense strand and the antisense strand independently further comprise at least one phosphorothioate or methylphosphonate internucleotide linkage, and wherein at least one strand is conjugated to a ligand.

In one embodiment, all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a modification selected from the group consisting of a 2'-O-methyl modification, a 2'-fluoro modification, and a 2'-deoxy-modification.

In one embodiment, the sense strand comprises 2-6, *e.g.*, 2, 3, 4, 5, or 6, 2'-fluoro modified nucleotides. In another embodiment, the sense strand comprises no more than 6, *e.g.*, 0, 1, 2, 3, 4, 5, or 6, 2'-fluoro modified nucleotides.

In one embodiment, the sense strand comprises no more than 2, *e.g.*, 0, 1, or 2, 2'-deoxy-modified nucleotides.

In one embodiment, the antisense strand comprises no more than 4, *e.g.*, 0, 1, 2, 3, or 4, 2'-fluoro modified nucleotides.

In one embodiment, the antisense strand comprises no more than 5, *e.g.*, 0, 1, 2, 3, 4, or 5, 2'-deoxy-modified nucleotides. In another embodiment, the antisense strand comprises 1-5, *e.g.*, 1, 2, 3, 4, or 5, deoxy-nucleotides.

In one embodiment, the sense strand comprises 4 2'-fluoro modified nucleotides, *e.g.*, nucleotides 7 and 9-11, counting from the 5'-end, and the antisense strand comprises 2 2'-fluoro modified nucleotides, *e.g.*, nucleotides 14 and 16, counting from the 5'-end, and 3 2'-deoxy-modified nucleotides, *e.g.*, nucleotides 2, 5, and 7, counting from the 5'-end.

In one embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at the 3'-terminus of one strand, *e.g.*, the antisense strand or the sense strand.

In another embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at the 5'-terminus of one strand, *e.g.*, the antisense strand or the sense strand.

In one embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at both the 5'- and 3'-terminus of one strand. In one embodiment, the strand is the antisense strand.

In one embodiment, the sense strand comprises two phosphorothioate or methylphosphonate internucleotide linkages at the 5'-terminus.

In one embodiment, the antisense strand comprises two phosphorothioate or methylphosphonate internucleotide linkages at the 5'-terminus.

In another embodiment, the antisense strand comprises two phosphorothioate or methylphosphonate internucleotide linkages at both the 5'- and 3'-terminus.

- 5 In one embodiment, the sense strand comprises two phosphorothioate or methylphosphonate internucleotide linkages at the 5'-terminus and the antisense strand comprises two phosphorothioate or methylphosphonate internucleotide linkages at both the 5'- and 3'-terminus.

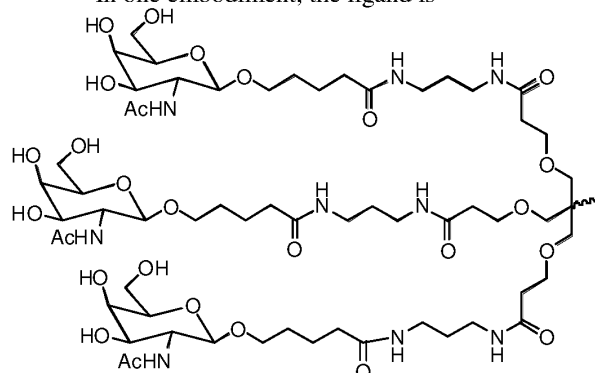
In one embodiment, the ligand is conjugated to the sense strand.

In one embodiment the ligand is conjugated to the 3'-end of the sense strand.

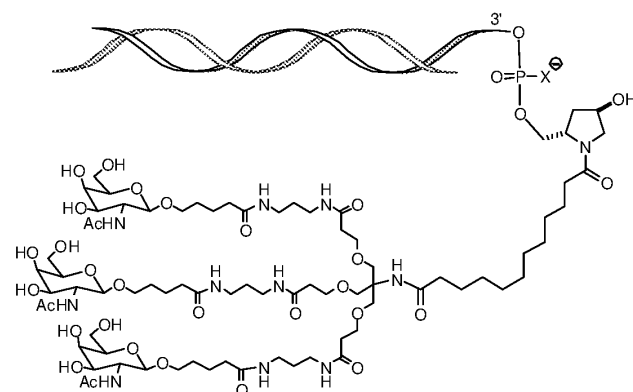
- 10 In one embodiment, the ligand is an N-acetylgalactosamine (GalNAc) derivative.

In one embodiment, the ligand is one or more GalNAc derivatives attached through a monovalent, bivalent, or trivalent branched linker.

In one embodiment, the ligand is



- 15 In one embodiment, the dsRNA agent is conjugated to the ligand as shown in the following schematic



and, wherein X is O or S.

In one embodiment, the X is O.

- 20 In one embodiment, the sense strand comprises at least 17, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of 5'-CUUAAAAGGGACAGUAUUCUA-3' (SEQ ID NO: 13).

In one embodiment, the sense strand comprises at least 19, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of 5'-CUUAAAAGGGACAGUAUUCUA-3' (SEQ ID NO: 13).

In one embodiment, the sense strand comprises the nucleotide sequence of 5'-
5 CUUAAAAGGGACAGUAUUCUA-3' (SEQ ID NO: 13).

In one embodiment, the antisense strand comprises at least 17, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of 5'-UAGAAUACUGUCCCUUUUAAGCC-3' (SEQ ID NO: 14).

In one embodiment, the antisense strand comprises at least 19, contiguous nucleotides
10 differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of 5'-UAGAAUACUGUCCCUUUUAAGCC-3' (SEQ ID NO: 14).

In one embodiment, the antisense strand comprises at least 21, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of 5'-UAGAAUACUGUCCCUUUUAAGCC-3' (SEQ ID NO: 14).

In one embodiment, the antisense strand comprises the nucleotide sequence of 5'-
15 UAGAAUACUGUCCCUUUUAAGCC-3' (SEQ ID NO: 14).

In one embodiment, the sense strand comprises the nucleotide sequence of 5'-CUUAAAAGGGACAGUAUUCUA-3' (SEQ ID NO: 13) and the antisense strand comprises the nucleotide sequence of 5'-UAGAAUACUGUCCCUUUUAAGCC-3' (SEQ ID NO: 14).

In one embodiment, the sense strand differs by no more than 3, *e.g.*, 0, 1, 2, or 3, modified nucleotides from the nucleotide sequence of 5'-csusuaaaAfgGfGfAfcagauucua-3' (SEQ ID NO: 15) wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U respectively; and s is a phosphorothioate linkage.

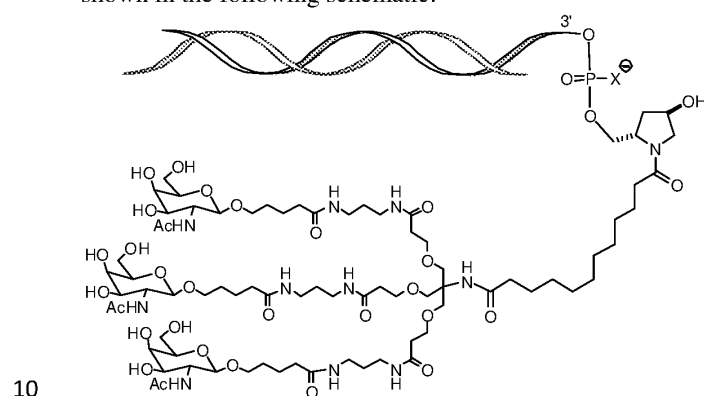
In one embodiment, the antisense strand differs by no more than 3, *e.g.*, 0, 1, 2, or 3, modified nucleotides from the nucleotide sequence of 5'-usdAsgadAudAcuguccCfuUfuuaagscsc-3' (SEQ ID NO: 16), wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U respectively; dA is a 2'-deoxyadenosine-3'-phosphate nucleotide; and s is a phosphorothioate linkage.

In one embodiment, the sense strand comprises the nucleotide sequence of 5'-csusuaaaAfgGfGfAfcagauucua-3' (SEQ ID NO: 15) and the antisense strand comprises the nucleotide sequence of 5'-usdAsgadAudAcuguccCfuUfuuaagscsc-3' (SEQ ID NO: 16), wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U respectively; dA is a 2'-deoxyadenosine-3'-phosphate nucleotide; and s is a phosphorothioate linkage.

In one embodiment, the sense strand comprises the nucleotide sequence of 5'-csusuaaaAfgGfGfAfcagauucuaL96-3' (SEQ ID NO: 17) and the antisense strand comprises the nucleotide sequence of 5'-usdAsgadAudAcuguccCfuUfuuaagscsc-3' (SEQ ID NO: 16), wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2'-fluoro A,

G, C and U respectively; dA is a 2'-deoxyadenosine-3'-phosphate nucleotide; s is a phosphorothioate linkage, and L96 is N-[tris(GalNAc-alkyl)-amidodecanoyl]-4-hydroxyprolinol.

- In one embodiment, the sense strand comprises the nucleotide sequence of 5'-csusuaaaAfgGfGfAfcagauucua-3' (SEQ ID NO: 15) and the antisense strand comprises the nucleotide sequence of 5'-usdAsgadAudAcuguccCfuUfuuaagscsc-3' (SEQ ID NO: 16, wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U respectively; dA is a 2'-deoxyadenosine-3'-phosphate nucleotide; and s is a phosphorothioate linkage, wherein the 3'-end of the sense strand is conjugated to the ligand as shown in the following schematic:



and, wherein X is O.

In one embodiment, the sense strand comprises at least 17, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of 5'-CUUAAAAGGGACAGUAUUCUU-3' (SEQ ID NO: 48).

- In one embodiment, the sense strand comprises at least 19, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of 5'-CUUAAAAGGGACAGUAUUCUU-3' (SEQ ID NO: 48).

In one embodiment, the sense strand comprises the nucleotide sequence of 5'-CUUAAAAGGGACAGUAUUCUU-3' (SEQ ID NO: 48).

- In one embodiment, the antisense strand comprises at least 17, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of 5'-AAGAAUACUGUCCCUUUUAAGCC-3' (SEQ ID NO: 315).

In one embodiment, the antisense strand comprises at least 19, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of 5'-

- AAGAAUACUGUCCCUUUUAAGCC-3' (SEQ ID NO: 315).

In one embodiment, the antisense strand comprises at least 21, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of 5'-AAGAAUACUGUCCCUUUUAAGCC-3' (SEQ ID NO: 315).

- In one embodiment, the antisense strand comprises the nucleotide sequence of 5'-AAGAAUACUGUCCCUUUUAAGCC-3' (SEQ ID NO: 315).

In one embodiment, the sense strand comprises the nucleotide sequence of 5'-CUUAAAAGGGACAGUAUUCUU-3' (SEQ ID NO: 48) and the antisense strand comprises the nucleotide sequence of 5'-AAGAAUACUGUCCCUUUUAAGCC-3' (SEQ ID NO: 315).

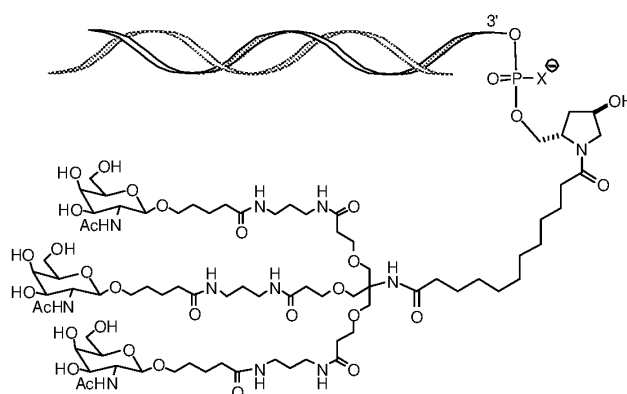
In one embodiment, the sense strand differs by no more than 3, e.g., 0, 1, 2, or 3, modified nucleotides from the nucleotide sequence of 5'-csusuaaaAfgGfGfAfcagauucuu-3' (SEQ ID NO: 377) wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U respectively; and s is a phosphorothioate linkage.

In one embodiment, the antisense strand differs by no more than 3, e.g., 0, 1, 2, or 3, modified nucleotides from the nucleotide sequence of 5'-asdAsgadAudAcuguccCfuUfuuaagscsc-3' (SEQ ID NO: 866), wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U respectively; dA is a 2'-deoxyadenosine-3'-phosphate nucleotide; and s is a phosphorothioate linkage.

In one embodiment, the sense strand comprises the nucleotide sequence of 5'-csusuaaaAfgGfGfAfcagauucuu-3' (SEQ ID NO: 377) and the antisense strand comprises the nucleotide sequence of 5'-asdAsgadAudAcuguccCfuUfuuaagscsc-3' (SEQ ID NO: 866), wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U respectively; dA is a 2'-deoxyadenosine-3'-phosphate nucleotide; and s is a phosphorothioate linkage.

In one embodiment, the sense strand comprises the nucleotide sequence of 5'-csusuaaaAfgGfGfAfcagauucuuL96-3' (SEQ ID NO: 377) and the antisense strand comprises the nucleotide sequence of 5'-asdAsgadAudAcuguccCfuUfuuaagscsc-3' (SEQ ID NO: 866), wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U respectively; dA is a 2'-deoxyadenosine-3'-phosphate nucleotide; s is a phosphorothioate linkage, and L96 is N-[tris(GalNAc-alkyl)-amidodecanoyl]-4-hydroxyprolinol.

In one embodiment, the sense strand comprises the nucleotide sequence of 5'-csusuaaaAfgGfGfAfcagauucuu-3' (SEQ ID NO: 377) and the antisense strand comprises the nucleotide sequence of 5'-asdAsgadAudAcuguccCfuUfuuaagscsc-3' (SEQ ID NO: 866), wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U respectively; dA is a 2'-deoxyadenosine-3'-phosphate nucleotide; and s is a phosphorothioate linkage, wherein the 3'-end of the sense strand is conjugated to the ligand as shown in the following schematic:



and, wherein X is O.

In one embodiment, the sense strand comprises at least 17, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of 5' -

5 AAAAGGGACAGUAUUCUCAGU -3' (SEQ ID NO: 30).

In one embodiment, the sense strand comprises at least 19, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of 5'-

AAAAGGGACAGUAUUCUCAGU -3' (SEO ID NO: 30).

In one embodiment, the sense strand comprises the nucleotide sequence of 5'-
10 AAAAGGGACAGUAUUCUCAGU -3' (SEQ ID NO: 30).

In one embodiment, the antisense strand comprises at least 17, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of 5'-ACUGAGAAUACUGUCCCUUUUAA -3' (SEQ ID NO: 31).

In one embodiment, the antisense strand comprises at least 19, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of 5'-ACUGAGAAUACUGUCCCUUUUAA -3' (SEQ ID NO: 31).

In one embodiment, the antisense strand comprises at least 21, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of 5'-ACUGAGAAUACUGUCCCUUUUAA -3' (SEQ ID NO: 31).

20 In one embodiment, the antisense strand comprises the nucleotide sequence of 5'-ACUGAGAAUACUGUCCCUUUUAA-3' (SEQ ID NO: 31).

In one embodiment, the sense strand comprises the nucleotide sequence of 5'-AAAAGGGACAGUAUUCUCAGU -3'(SEQ ID NO: 30) and the antisense strand comprises the nucleotide sequence of 5'- ACUGAGAAUACUGUCCCUUUUAA -3' (SEQ ID NO: 31).

25 In one embodiment, the sense strand differs by no more than 3, e.g., 0, 1, 2, or 3, modified nucleotides from the nucleotide sequence of 5'- asasaaggGfAfGfuaucucagu -3' (SEQ ID NO: 350) wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U respectively; and s is a phosphorothioate linkage.

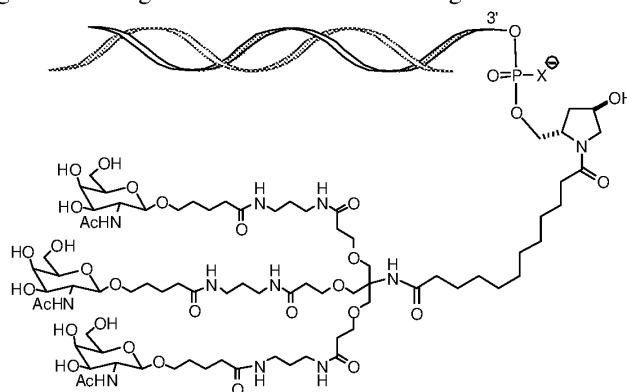
In one embodiment, the antisense strand differs by no more than 3, e.g., 0, 1, 2, or 3, modified
30 nucleotides from the nucleotide sequence of 5'- asCfsugaGfaAfUfacugUfcCfcuuuusasa -3' (SEQ ID

NO: 351), wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U respectively; and s is a phosphorothioate linkage.

In one embodiment, the sense strand comprises the nucleotide sequence of 5'-asasaaggGfaCfAfGfuaucucagu-3' (SEQ ID NO: 350) and the antisense strand comprises the nucleotide sequence of 5'-asCfsugaGfaAfUfacugUfcCfcuuusasa-3' (SEQ ID NO: 351), wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U respectively; and s is a phosphorothioate linkage.

In one embodiment, the sense strand comprises the nucleotide sequence of 5'-asasaaggGfaCfAfGfuaucucaguL96-3' (SEQ ID NO: 350) and the antisense strand comprises the nucleotide sequence of 5'-asCfsugaGfaAfUfacugUfcCfcuuusasa-3' (SEQ ID NO: 351), wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U respectively; s is a phosphorothioate linkage, and L96 is N-[tris(GalNAc-alkyl)-amidodecanoyl]-4-hydroxyprolinol.

In one embodiment, the sense strand comprises the nucleotide sequence of 5'-asasaaggGfaCfAfGfuaucucagu-3' (SEQ ID NO: 350) and the antisense strand comprises the nucleotide sequence of 5'-asCfsugaGfaAfUfacugUfcCfcuuusasa-3' (SEQ ID NO: 351), wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U respectively; and s is a phosphorothioate linkage, wherein the 3'-end of the sense strand is conjugated to the ligand as shown in the following schematic:



and, wherein X is O.

In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) for inhibiting expression of apolipoprotein C3 in a cell, wherein the dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of nucleotides 429-456 of the nucleotide sequence of SEQ ID NO:1, and the antisense strand comprises at least 19 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2, wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand comprise a modification selected from the group consisting of a 2'-O-methyl modification, a 2'-fluoro modification, and a deoxy-modification, wherein both the

sense strand and the antisense strand independently further comprise at least one phosphorothioate or methylphosphonate internucleotide linkage, and wherein at least one strand is conjugated to a ligand.

In one embodiment, the sense strand comprises 2-6 2'-fluoro modified nucleotides.

In one embodiment, the antisense strand comprises no more than 4 2'-fluoro modified
5 nucleotides.

In one embodiment, the antisense strand comprises 1-5 deoxy-modified nucleotides.

In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of apolipoprotein C3 in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand
10 comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of nucleotides 429-456 of the nucleotide sequence of SEQ ID NO:1, and the antisense strand comprises at least 19 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2, wherein all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a modification selected from the group consisting of a 2'-O-methyl
15 modification, a 2'-fluoro modification, and a deoxy-modification, wherein both the sense strand and the antisense strand independently further comprise at least one phosphorothioate or methylphosphonate internucleotide linkage, and wherein at least one strand is conjugated to a ligand.

In one embodiment, the sense strand comprises 2-6 2'-fluoro modified nucleotides.

In one embodiment, the sense strand comprises 4 2'-fluoro modified nucleotides.

In one embodiment, the antisense strand comprises 2-4 2'-fluoro modified nucleotides.
20

In one embodiment, the antisense strand comprises 2 2'-fluoro modified nucleotides.

In one embodiment, the antisense strand comprises 1-5 2'-deoxy-modified nucleotides.

In one embodiment, the antisense strand comprises 3 2'-deoxy-modified nucleotides.

In one embodiment, the sense strand comprises 4 2'-fluoro modified nucleotides at
25 nucleotides 7 and 9-11, counting from the 5'-end, and the antisense strand comprises 2 2'-fluoro modified nucleotides at nucleotides 14 and 16, counting from the 5'-end, and 3 2'-deoxy-modified nucleotides at nucleotides 2, 5, and 7, counting from the 5'-end.

In one embodiment, the sense strand comprises two phosphorothioate or methylphosphonate internucleotide linkages at the 5'-terminus.

In one embodiment, the antisense strand comprises two phosphorothioate or
30 methylphosphonate internucleotide linkages at both the 5'- and the 3'-terminus.

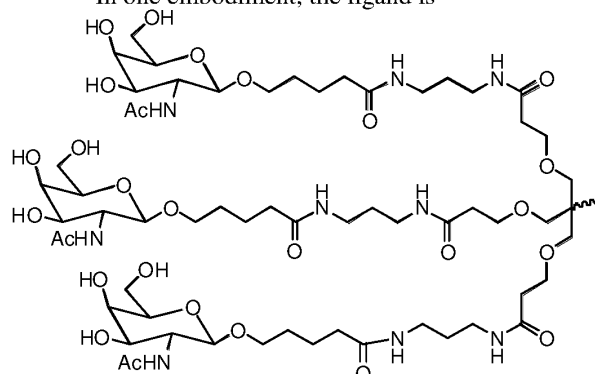
In one embodiment, the sense strand comprises two phosphorothioate or methylphosphonate internucleotide linkages at the 5'-terminus and the antisense strand comprises two phosphorothioate or methylphosphonate internucleotide linkages at both the 5'- and the 3'-terminus.

In one embodiment, the ligand is conjugated to the 3'-end of the sense strand.
35

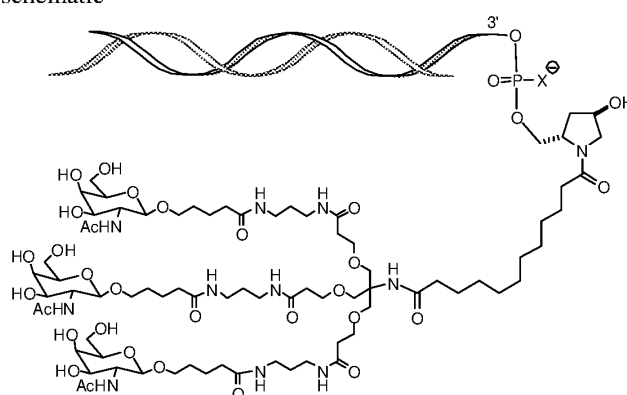
In one embodiment, the ligand is an N-acetylgalactosamine (GalNAc) derivative.

In one embodiment, the ligand is one or more GalNAc derivatives attached through a monovalent, bivalent, or trivalent branched linker.

In one embodiment, the ligand is



In one embodiment, the dsRNA agent is conjugated to the ligand as shown in the following schematic



5

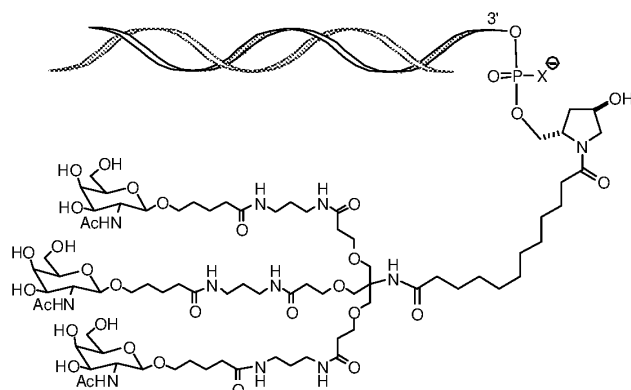
and, wherein X is O.

In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of apolipoprotein C3 in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand differs by no more than 3 modified nucleotides from the nucleotide sequence of 5'-
 10 csusuaaaAfgGfGfAfcagauucua-3' (SEQ ID NO:15) and wherein the antisense strand differs by no more than 3 modified nucleotides from the nucleotide sequence of 5'-
 usdAsgadAudAcuguccCfuUfuuaagscsc -3' (SEQ ID NO:16), wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U respectively;
 15 dA is a 2'-deoxyadenosine-3'-phosphate nucleotide; and s is a phosphorothioate linkage.

In one embodiment, the sense strand differs by no more than 2 modified nucleotides from the nucleotide sequence of 5'- csusuaaaAfgGfGfAfcagauucua-3' (SEQ ID NO:15) and wherein the antisense strand differs by no more than 2 modified nucleotides from the nucleotide sequence of 5'-
 usdAsgadAudAcuguccCfuUfuuaagscsc -3' (SEQ ID NO:16).

20 In one embodiment, the sense strand differs by no more than 1 modified nucleotide from the nucleotide sequence of 5'- csusuaaaAfgGfGfAfcagauucua-3' (SEQ ID NO:15) and wherein the antisense strand differs by no more than 1 modified nucleotides from the nucleotide sequence of 5'-
 usdAsgadAudAcuguccCfuUfuuaagscsc -3' (SEQ ID NO:16).

In one embodiment, the dsRNA agent is conjugated to the ligand as shown in the following schematic

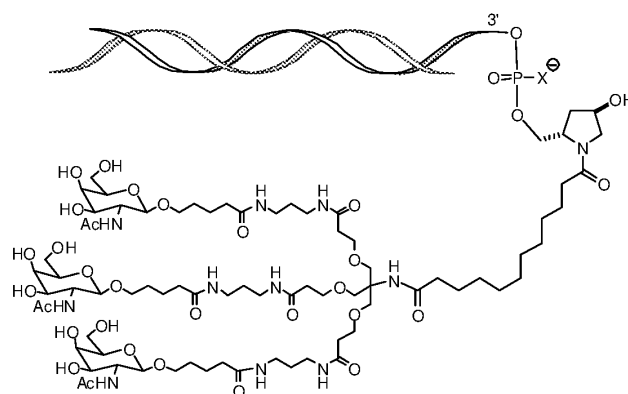


and, wherein X is O.

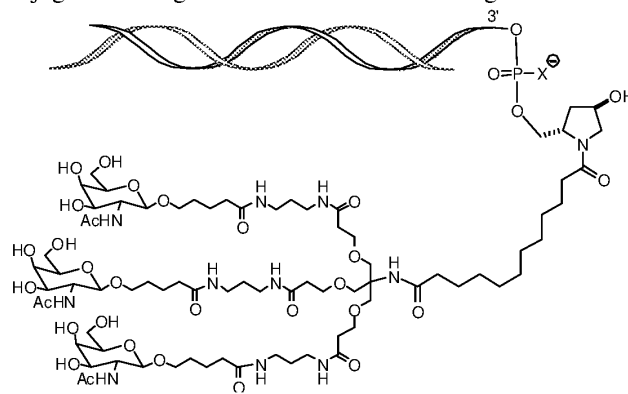
- 5 In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of apolipoprotein C3 in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises the nucleotide sequence of 5'-csusuaaaAfgGfGfAfcagauucua-3' (SEQ ID NO:15) and the antisense strand comprises the nucleotide sequence of 5'-usdAsgadAudAcuguccCfuUfuuaagscsc-3' *SEQ ID NO:16), wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U respectively; dA is a 2'-deoxyadenosine-3'-phosphate nucleotide; and s is a phosphorothioate linkage.

- 15 In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of apolipoprotein C3 in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises the nucleotide sequence of 5'-csusuaaaAfgGfGfAfcagauucuaL96-3' (SEQ ID NO:17) and the antisense strand comprises the nucleotide sequence of 5'-usdAsgadAudAcuguccCfuUfuuaagscsc-3' (SEQ ID NO:16), wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U respectively; dA is a 2'-deoxyadenosine-3'-phosphate nucleotide; s is a phosphorothioate linkage, and L96 is N-[tris(GalNAc-alkyl)-amidodecanoyl]-4-hydroxyprolinol.

- 20 In another aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of apolipoprotein C3 in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises the nucleotide sequence of 5'-csusuaaaAfgGfGfAfcagauucua-3' (SEQ ID NO:15) and the antisense strand comprises the nucleotide sequence of 5'-usdAsgadAudAcuguccCfuUfuuaagscsc-3' (SEQ ID NO:16), wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U respectively; dA is a 2'-deoxyadenosine-3'-phosphate nucleotide; and s is a phosphorothioate linkage; and wherein the 3'-end of the sense strand is
- 30 conjugated to a ligand as shown in the following schematic:



- In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of apolipoprotein C3 in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand
- 5 consists of the nucleotide sequence of 5'- csusuaaaAfgGfGfAfcagauucua-3' (SEQ ID NO:15) and the antisense strand consists of the nucleotide sequence of 5'- usdAsgadAudAcuguccCfuUfuuaagscsc-3' (SEQ ID NO:16), wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2'-fluoro A, G, C and U respectively; dA is a 2'-deoxyadenosine-3'-phosphate nucleotide; and s is a phosphorothioate linkage; and wherein the 3'-end of the sense strand is
- 10 conjugated to a ligand as shown in the following schematic:



The present invention also provides cells containing any of the dsRNA agents of the invention and pharmaceutical compositions comprising any of the dsRNA agents of the invention.

- The pharmaceutical composition of the invention may include dsRNA agent in an
- 15 unbuffered solution, *e.g.*, saline or water, or the pharmaceutical composition of the invention may include the dsRNA agent is in a buffer solution, *e.g.*, a buffer solution comprising acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof; or phosphate buffered saline (PBS).

- In one aspect, the present invention provides a method of inhibiting expression of an apolipoprotein C3 (APOC3) gene in a cell. The method includes contacting the cell with any of the
- 20 dsRNAs of the invention or any of the pharmaceutical compositions of the invention, thereby inhibiting expression of the APOC3 gene in the cell.

In one embodiment, the cell is within a subject, *e.g.*, a human subject, *e.g.*, a subject having an apolipoprotein C3-associated disorder, such as an apolipoprotein C3-associated disorder selected from the group consisting of hypertriglyceridemia, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, polycystic ovary syndrome, kidney disease, obesity, type 2 diabetes mellitus (insulin resistance), hypertension, atherosclerosis and pancreatitis.

In one embodiment, contacting the cell with the dsRNA agent inhibits the expression of APOC3 by at least 50%, 60%, 70%, 80%, 90%, or 95%.

In one embodiment, inhibiting expression of apolipoprotein C3 decreases APOC3 protein level in serum of the subject by at least 50%, 60%, 70%, 80%, 90%, or 95%.

In one aspect, the present invention provides a method of treating a subject having a disorder that would benefit from reduction in apolipoprotein C3 (APOC3) expression. The method includes administering to the subject a therapeutically effective amount of any of the dsRNAs of the invention or any of the pharmaceutical compositions of the invention, thereby treating the subject having the disorder that would benefit from reduction in APOC3 expression.

In another aspect, the present invention provides a method of preventing at least one symptom in a subject having a disorder that would benefit from reduction in apolipoprotein C3 (APOC3) expression. The method includes administering to the subject a prophylactically effective amount of any of the dsRNAs of the invention or any of the pharmaceutical compositions of the invention, thereby preventing at least one symptom in the subject having the disorder that would benefit from reduction in APOC3 expression.

In one embodiment, the disorder is an apolipoprotein C3-associated disorder, *e.g.*, an apolipoprotein C3-associated disorder is selected from the group consisting of hypertriglyceridemia, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, polycystic ovary syndrome, kidney disease, obesity, type 2 diabetes mellitus (insulin resistance), hypertension, atherosclerosis and pancreatitis.

In one embodiment, the apolipoprotein C3-associated disorder is hypertriglyceridemia.

In one embodiment, the subject is human.

In one embodiment, the dsRNA agent is administered to the subject at a dose of about 0.01 mg/kg to about 50 mg/kg.

In one embodiment, the dsRNA agent is administered to the subject subcutaneously.

In one embodiment, the methods of the invention include further determining the level of apolipoprotein C3 in a sample(s) from the subject.

In one embodiment, the level of apolipoprotein C3 in the subject sample(s) is an apolipoprotein C3 protein level in a blood or serum sample(s).

In certain embodiments, the methods of the invention further comprise administering to the subject an additional therapeutic agent. In one embodiment, the additional therapeutic is a dsRNA agent targeting PCSK9, *e.g.*, Inclisiran. In one embodiment, the additional therapeutic is a PCSK9 inhibitor. In one embodiment, the PCSK9 inhibitor is an anti-PCSK9 monoclonal antibody, *e.g.*, evolocumab (Repatha®) and alirocumab (Praluent®). In another embodiment, the PCSK9 inhibitor is

a dsRNA agent targeting PCSK9, *e.g.*, Inclisiran. In a further embodiment, the additional therapeutic agent is selected from the group consisting of an HMG-CoA reductase inhibitor, a fibrate, a bile acid sequestrant, niacin, an antiplatelet agent, an angiotensin converting enzyme inhibitor, an angiotensin II receptor antagonist, an acylCoA cholesterol acetyltransferase (ACAT) inhibitor, a cholesterol absorption inhibitor, a cholesterol ester transfer protein (CETP) inhibitor, a microsomal triglyceride transfer protein (MTTP) inhibitor, a cholesterol modulator, a bile acid modulator, a peroxisome proliferation activated receptor (PPAR) agonist, a gene-based therapy, a composite vascular protectant, a glycoprotein IIb/IIIa inhibitor, aspirin or an aspirin-like compound, an IBAT inhibitor, a squalene synthase inhibitor, a monocyte chemoattractant protein (MCP)-I inhibitor, or fish oil.

The present invention also provides kits comprising any of the dsRNAs of the invention or any of the pharmaceutical compositions of the invention, and optionally, instructions for use.

Brief Description of the Drawings

Figure 1 is a graph showing human APOC3 mRNA levels in mice (n = 3 per group) subcutaneously administered a single 3 mg/kg dose of the indicated dsRNA duplexes, on day14 post-dose. Human APOC3 mRNA levels are shown relative to control levels detected with PBS treatment.

Figure 2 is a graph showing human APOC3 mRNA levels in mice (n = 3 per group) subcutaneously administered a single 3 mg/kg dose of the indicated dsRNA duplexes, on day14 post-dose. Human APOC3 mRNA levels are shown relative to control levels detected with PBS treatment.

Figure 3 is a graph showing human APOC3 mRNA levels in mice (n = 3 per group) subcutaneously administered a single 3 mg/kg dose of the indicated dsRNA duplexes, on day14 post-dose. Human APOC3 mRNA levels are shown relative to control levels detected with PBS treatment.

Detailed Description of the Invention

The present invention provides iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of an apolipoprotein C3 (APO3) gene. The gene may be within a cell, *e.g.*, a cell within a subject, such as a human. The use of these iRNAs enables the targeted degradation of mRNAs of the corresponding gene (apolipoprotein C3 gene) in mammals.

The iRNAs of the invention have been designed to target the human apolipoprotein C3 gene, including portions of the gene that are conserved in the apolipoprotein C3 orthologs of other mammalian species. Without intending to be limited by theory, it is believed that a combination or sub-combination of the foregoing properties and the specific target sites or the specific modifications in these iRNAs confer to the iRNAs of the invention improved efficacy, stability, potency, durability, and safety.

Accordingly, the present invention provides methods for treating and preventing an apolipoprotein C3-associated disorder, *e.g.*, hypertriglyceridemia, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, polycystic ovary syndrome, kidney disease, obesity, type 2 diabetes mellitus (insulin resistance), hypertension, arteriosclerosis and pancreatitis, using iRNA

compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a apolipoprotein C3 gene.

The iRNAs of the invention include an RNA strand (the antisense strand) having a region which is up to about 30 nucleotides or less in length, *e.g.*, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length, which region is substantially complementary to at least part of an mRNA transcript of an APOC3 gene.

In certain embodiments, one or both of the strands of the double stranded RNAi agents of the invention is up to 66 nucleotides in length, *e.g.*, 36-66, 26-36, 25-36, 31-60, 22-43, 27-53 nucleotides in length, with a region of at least 19 contiguous nucleotides that is substantially complementary to at least a part of an mRNA transcript of an APOC3 gene. In some embodiments, such iRNA agents having longer length antisense strands preferably may include a second RNA strand (the sense strand) of 20-60 nucleotides in length wherein the sense and antisense strands form a duplex of 18-30 contiguous nucleotides.

The use of iRNAs of the invention enables the targeted degradation of mRNAs of the corresponding gene (apolipoprotein C3 gene) in mammals. Using *in vitro* assays, the present inventors have demonstrated that iRNAs targeting an APOC3 gene can potently mediate RNAi, resulting in significant inhibition of expression of an APOC3 gene. Thus, methods and compositions including these iRNAs are useful for treating a subject having an apolipoprotein C3-associated disorder, *e.g.*, hypertriglyceridemia, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, polycystic ovary syndrome, kidney disease, obesity, type 2 diabetes mellitus (insulin resistance), hypertension, atherosclerosis and pancreatitis.

Accordingly, the present invention provides methods and combination therapies for treating a subject having a disorder that would benefit from inhibiting or reducing the expression of an APOC3 gene, *e.g.*, an apolipoprotein C3-associated disease, such as hypertriglyceridemia, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, polycystic ovary syndrome, kidney disease, obesity, type 2 diabetes mellitus (insulin resistance), hypertension, atherosclerosis and pancreatitis, using iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of an APOC3 gene.

The present invention also provides methods for preventing at least one symptom in a subject having a disorder that would benefit from inhibiting or reducing the expression of an APOC3 gene, *e.g.*, hypertriglyceridemia, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, polycystic ovary syndrome, kidney disease, obesity, type 2 diabetes mellitus (insulin resistance), hypertension, atherosclerosis and pancreatitis. For example, in a subject having hypertriglyceridemia, the methods of the present invention may reduce at least one symptom in the subject, *e.g.*, lower triglyceride levels.

The following detailed description discloses how to make and use compositions containing iRNAs to inhibit the expression of an APOC3 gene as well as compositions, uses, and methods for

treating subjects that would benefit from inhibition and/or reduction of the expression of an APOC3 gene, *e.g.*, subjects susceptible to or diagnosed with an apolipoprotein C3-associated disorder.

I. Definitions

5 In order that the present invention may be more readily understood, certain terms are first defined. In addition, it should be noted that whenever a value or range of values of a parameter are recited, it is intended that values and ranges intermediate to the recited values are also intended to be part of this invention.

The articles "a" and "an" are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, "an element" means one element or
10 more than one element, *e.g.*, a plurality of elements.

The term "including" is used herein to mean, and is used interchangeably with, the phrase "including but not limited to".

The term "or" is used herein to mean, and is used interchangeably with, the term "and/or,"
15 unless context clearly indicates otherwise. For example, "sense strand or antisense strand" is understood as "sense strand or antisense strand or sense strand and antisense strand."

The term "about" is used herein to mean within the typical ranges of tolerances in the art. For example, "about" can be understood as about 2 standard deviations from the mean. In certain embodiments, about means $\pm 10\%$. In certain embodiments, about means $\pm 5\%$. When about is
20 present before a series of numbers or a range, it is understood that "about" can modify each of the numbers in the series or range.

The term "at least" prior to a number or series of numbers is understood to include the number adjacent to the term "at least", and all subsequent numbers or integers that could logically be included, as clear from context. For example, the number of nucleotides in a nucleic acid molecule
25 must be an integer. For example, "at least 19 nucleotides of a 21 nucleotide nucleic acid molecule" means that 19, 20, or 21 nucleotides have the indicated property. When at least is present before a series of numbers or a range, it is understood that "at least" can modify each of the numbers in the series or range.

As used herein, "no more than" or "less than" is understood as the value adjacent to the
30 phrase and logical lower values or integers, as logical from context, to zero. For example, a duplex with an overhang of "no more than 2 nucleotides" has a 2, 1, or 0 nucleotide overhang. When "no more than" is present before a series of numbers or a range, it is understood that "no more than" can modify each of the numbers in the series or range. As used herein, ranges include both the upper and lower limit.

35 As used herein, methods of detection can include determination that the amount of analyte present is below the level of detection of the method.

In the event of a conflict between an indicated target site and the nucleotide sequence for a sense or antisense strand, the indicated sequence takes precedence.

In the event of a conflict between a sequence and its indicated site on a transcript or other sequence, the nucleotide sequence recited in the specification takes precedence.

As used herein, the term “APOC3” refers to the well-known gene that encodes apolipoprotein C3, as well as to its protein product, also known in the art as HALP2 or APOCIII.

5 The term “APOC3” includes human APOC3, the amino acid and complete coding sequence of which may be found in for example, GenBank Accession No. GI:4557322 (NM_000040.3; SEQ ID NO:1; reverse complement, SEQ ID NO:2); *Macaca fascicularis* APOC3, the amino acid and complete coding sequence of which may be found in for example, GenBank Accession No. GI:544489959 (XM_05579730.1, SEQ ID NO:3; reverse complement, SEQ ID NO:4); *Macaca*
10 *mulatta* APOC3, the amino acid and complete coding sequence of which may be found in for example, GenBank Accession No. GI:297269260 (XM_001090312.4; SEQ ID NO: 5; reverse complement, SEQ ID NO:6); mouse (*Mus musculus*) APOC3, the amino acid and complete coding sequence of which may be found in for example, GenBank Accession No. GI:577019555 (NM_023114.4, SEQ ID NO:7; reverse complement, SEQ ID NO:8); rat (*Rattus norvegicus*) APOC3,
15 the amino acid and complete coding sequence of which may be found in for example, GenBank Accession No. GI:402534545 (NM_012501.2, SEQ ID NO:9; reverse complement, SEQ ID NO:2-10); and rabbit (*Oryctolagus cuniculus*), GenBank Accession No. GI:655601498 (XM_002708371.3, SEQ ID NO:11; reverse complement, SEQ ID NO:12).

Further information on APOC3 can be found, for example, at
20 www.ncbi.nlm.nih.gov/gene/345.

Additional examples of APOC3 mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, and the *Macaca* genome project web site.

The term “APOC3,” as used herein, also refers to naturally occurring DNA sequence variations of the APOC3 gene, such as a single nucleotide polymorphism (SNP) in the APOC3 gene.
25 Exemplary SNPs in the APOC3 DNA sequence may be found through the dbSNP database available at www.ncbi.nlm.nih.gov/projects/SNP/. Non-limiting examples of sequence variations within the APOC3 gene include, for example, the two variations rs2854116 and rs2854117, described in Petersen, K.F. *et al.*, (2010), *N. Engl. J. Med.* 362(12):1082-1089, the entire contents of which are incorporated herein by reference.

30 Exemplary APOC3 nucleotide sequences may also be found in SEQ ID NOs:1-12. SEQ ID NOs:2, 4, 6, 8, 10, and 12 are the reverse complement sequences of SEQ ID NOs:1, 3, 5, 7, 9, and 11, respectively.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application. As used
35 herein, “target sequence” refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of an apolipoprotein C3 gene, including mRNA that is a product of RNA processing of a primary transcription product. The target portion of the sequence will be at least long enough to serve as a substrate for iRNA-directed cleavage at or near that portion

of the nucleotide sequence of an mRNA molecule formed during the transcription of an APOC3 gene. In one embodiment, the target sequence is within the protein coding region of APOC3.

The target sequence may be from about 19-36 nucleotides in length, *e.g.*, preferably about 19-30 nucleotides in length. For example, the target sequence can be about 19-30 nucleotides, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length. In certain embodiments, the target sequence is 19-23 nucleotides in length, optionally 21-23 nucleotides in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the disclosure.

As used herein, the term “strand comprising a sequence” refers to an oligonucleotide comprising a chain of nucleotides that is described by the sequence referred to using the standard nucleotide nomenclature.

“G,” “C,” “A,” “T,” and “U” each generally stand for a nucleotide that contains guanine, cytosine, adenine, thymidine, and uracil as a base, respectively. However, it will be understood that the term “ribonucleotide” or “nucleotide” can also refer to a modified nucleotide, as further detailed below, or a surrogate replacement moiety (see, *e.g.*, Table 1). The skilled person is well aware that guanine, cytosine, adenine, and uracil can be replaced by other moieties without substantially altering the base pairing properties of an oligonucleotide comprising a nucleotide bearing such replacement moiety. For example, without limitation, a nucleotide comprising inosine as its base can base pair with nucleotides containing adenine, cytosine, or uracil. Hence, nucleotides containing uracil, guanine, or adenine can be replaced in the nucleotide sequences of dsRNA featured in the invention by a nucleotide containing, for example, inosine. In another example, adenine and cytosine anywhere in the oligonucleotide can be replaced with guanine and uracil, respectively to form G-U Wobble base pairing with the target mRNA. Sequences containing such replacement moieties are suitable for the compositions and methods featured in the invention.

The terms “iRNA”, “RNAi agent,” “iRNA agent,” “RNA interference agent” as used interchangeably herein, refer to an agent that contains RNA as that term is defined herein, and which mediates the targeted cleavage of an RNA transcript *via* an RNA-induced silencing complex (RISC) pathway. iRNA directs the sequence-specific degradation of mRNA through a process known as RNA interference (RNAi). The iRNA modulates, *e.g.*, inhibits, the expression of an apolipoprotein C3 gene in a cell, *e.g.*, a cell within a subject, such as a mammalian subject.

In one embodiment, an RNAi agent of the invention includes a single stranded RNA that interacts with a target RNA sequence, *e.g.*, an apolipoprotein C3 target mRNA sequence, to direct the cleavage of the target RNA. Without wishing to be bound by theory it is believed that long double stranded RNA introduced into cells is broken down into siRNA by a Type III endonuclease known as Dicer (Sharp *et al.* (2001) *Genes Dev.* 15:485). Dicer, a ribonuclease-III-like enzyme, processes the dsRNA into 19-23 base pair short interfering RNAs with characteristic two base 3' overhangs (Bernstein, *et al.*, (2001) *Nature* 409:363). The siRNAs are then incorporated into an RNA-induced silencing complex (RISC) where one or more helicases unwind the siRNA duplex, enabling the

complementary antisense strand to guide target recognition (Nykanen, *et al.*, (2001) *Cell* 107:309). Upon binding to the appropriate target mRNA, one or more endonucleases within the RISC cleave the target to induce silencing (Elbashir, *et al.*, (2001) *Genes Dev.* 15:188). Thus, in one aspect the invention relates to a single stranded RNA (siRNA) generated within a cell and which promotes the formation of a RISC complex to effect silencing of the target gene, *i.e.*, an apolipoprotein C3 (APOC3) gene. Accordingly, the term “siRNA” is also used herein to refer to an iRNA as described above.

In certain embodiments, the RNAi agent may be a single-stranded siRNA (ssRNAi) that is introduced into a cell or organism to inhibit a target mRNA. Single-stranded RNAi agents bind to the RISC endonuclease, Argonaute 2, which then cleaves the target mRNA. The single-stranded siRNAs are generally 15-30 nucleotides and are chemically modified. The design and testing of single-stranded siRNAs are described in U.S. Patent No. 8,101,348 and in Lima *et al.*, (2012) *Cell* 150:883-894, the entire contents of each of which are hereby incorporated herein by reference. Any of the antisense nucleotide sequences described herein may be used as a single-stranded siRNA as described herein or as chemically modified by the methods described in Lima *et al.*, (2012) *Cell* 150:883-894.

In certain embodiments, an “iRNA” for use in the compositions, uses, and methods of the invention is a double stranded RNA and is referred to herein as a “double stranded RNA agent,” “double stranded RNA (dsRNA) molecule,” “dsRNA agent,” or “dsRNA”. The term “dsRNA”, refers to a complex of ribonucleic acid molecules, having a duplex structure comprising two anti-parallel and substantially complementary nucleic acid strands, referred to as having “sense” and “antisense” orientations with respect to a target RNA, *i.e.*, an apolipoprotein C3 (APOC3) gene. In some embodiments of the invention, a double stranded RNA (dsRNA) triggers the degradation of a target RNA, *e.g.*, an mRNA, through a post-transcriptional gene-silencing mechanism referred to herein as RNA interference or RNAi.

In general, the majority of nucleotides of each strand of a dsRNA molecule are ribonucleotides, but as described in detail herein, each or both strands can also include one or more non-ribonucleotides, *e.g.*, a deoxyribonucleotide or a modified nucleotide. In addition, as used in this specification, an “iRNA” may include ribonucleotides with chemical modifications; an iRNA may include substantial modifications at multiple nucleotides. As used herein, the term “modified nucleotide” refers to a nucleotide having, independently, a modified sugar moiety, a modified internucleotide linkage, or modified nucleobase, or any combination thereof. Thus, the term modified nucleotide encompasses substitutions, additions or removal of, *e.g.*, a functional group or atom, to internucleoside linkages, sugar moieties, or nucleobases. The modifications suitable for use in the agents of the invention include all types of modifications disclosed herein or known in the art. Any such modifications, as used in a siRNA type molecule, are encompassed by “iRNA” or “RNAi agent” for the purposes of this specification and claims.

In certain embodiments of the instant disclosure, inclusion of a deoxy-nucleotide if present within an RNAi agent can be considered to constitute a modified nucleotide.

The duplex region may be of any length that permits specific degradation of a desired target RNA through a RISC pathway, and may range from about 19 to 36 base pairs in length, *e.g.*, about 19-30 base pairs in length, for example, about 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36 base pairs in length, such as about 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs in length. In certain embodiments, the duplex region is 19-21 base pairs in length, *e.g.*, 21 base pairs in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the disclosure.

The two strands forming the duplex structure may be different portions of one larger RNA molecule, or they may be separate RNA molecules. Where the two strands are part of one larger molecule, and therefore are connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure, the connecting RNA chain is referred to as a "hairpin loop." A hairpin loop can comprise at least one unpaired nucleotide. In some embodiments, the hairpin loop can comprise at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 23 or more unpaired nucleotides. In some embodiments, the hairpin loop can be 10 or fewer nucleotides. In some embodiments, the hairpin loop can be 8 or fewer unpaired nucleotides. In some embodiments, the hairpin loop can be 4-10 unpaired nucleotides. In some embodiments, the hairpin loop can be 4-8 nucleotides.

Where the two substantially complementary strands of a dsRNA are comprised by separate RNA molecules, those molecules need not be, but can be covalently connected. Where the two strands are connected covalently by means other than an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure, the connecting structure is referred to as a "linker." The RNA strands may have the same or a different number of nucleotides. The maximum number of base pairs is the number of nucleotides in the shortest strand of the dsRNA minus any overhangs that are present in the duplex. In addition to the duplex structure, an RNAi may comprise one or more nucleotide overhangs. In one embodiment of the RNAi agent, at least one strand comprises a 3' overhang of at least 1 nucleotide. In another embodiment, at least one strand comprises a 3' overhang of at least 2 nucleotides, *e.g.*, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In other embodiments, at least one strand of the RNAi agent comprises a 5' overhang of at least 1 nucleotide. In certain embodiments, at least one strand comprises a 5' overhang of at least 2 nucleotides, *e.g.*, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In still other embodiments, both the 3' and the 5' end of one strand of the RNAi agent comprise an overhang of at least 1 nucleotide.

In certain embodiments, an iRNA agent of the invention is a dsRNA, each strand of which comprises 19-23 nucleotides, that interacts with a target RNA sequence, *e.g.*, an apolipoprotein C3 (APOC3) gene, to direct cleavage of the target RNA.

In some embodiments, an iRNA of the invention is a dsRNA of 24-30 nucleotides that interacts with a target RNA sequence, *e.g.*, an APOC3 target mRNA sequence, to direct the cleavage of the target RNA.

As used herein, the term “nucleotide overhang” refers to at least one unpaired nucleotide that protrudes from the duplex structure of a double stranded iRNA. For example, when a 3'-end of one strand of a dsRNA extends beyond the 5'-end of the other strand, or *vice versa*, there is a nucleotide overhang. A dsRNA can comprise an overhang of at least one nucleotide; alternatively the overhang can comprise at least two nucleotides, at least three nucleotides, at least four nucleotides, at least five nucleotides or more. A nucleotide overhang can comprise or consist of a nucleotide/nucleoside analog, including a deoxynucleotide/nucleoside. The overhang(s) can be on the sense strand, the antisense strand, or any combination thereof. Furthermore, the nucleotide(s) of an overhang can be present on the 5'-end, 3'-end, or both ends of either an antisense or sense strand of a dsRNA.

In one embodiment, the antisense strand of a dsRNA has a 1-10 nucleotide, *e.g.*, a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide, overhang at the 3'-end or the 5'-end. In one embodiment, the sense strand of a dsRNA has a 1-10 nucleotide, *e.g.*, a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide, overhang at the 3'-end or the 5'-end. In another embodiment, one or more of the nucleotides in the overhang is replaced with a nucleoside thiophosphate.

In certain embodiments, the antisense strand of a dsRNA has a 1-10 nucleotide, *e.g.*, 0-3, 1-3, 2-4, 2-5, 4-10, 5-10, *e.g.*, a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide, overhang at the 3'-end or the 5'-end. In one embodiment, the sense strand of a dsRNA has a 1-10 nucleotide, *e.g.*, a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide, overhang at the 3'-end or the 5'-end. In another embodiment, one or more of the nucleotides in the overhang is replaced with a nucleoside thiophosphate.

In certain embodiments, the antisense strand of a dsRNA has a 1-10 nucleotides, *e.g.*, a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide, overhang at the 3'-end or the 5'-end. In certain embodiments, the overhang on the sense strand or the antisense strand, or both, can include extended lengths longer than 10 nucleotides, *e.g.*, 1-30 nucleotides, 2-30 nucleotides, 10-30 nucleotides, 10-25 nucleotides, 10-20 nucleotides, or 10-15 nucleotides in length. In certain embodiments, an extended overhang is on the sense strand of the duplex. In certain embodiments, an extended overhang is present on the 3' end of the sense strand of the duplex. In certain embodiments, an extended overhang is present on the 5' end of the sense strand of the duplex. In certain embodiments, an extended overhang is on the antisense strand of the duplex. In certain embodiments, an extended overhang is present on the 3' end of the antisense strand of the duplex. In certain embodiments, an extended overhang is present on the 5' end of the antisense strand of the duplex. In certain embodiments, one or more of the nucleotides in the extended overhang is replaced with a nucleoside thiophosphate. In certain embodiments, the overhang includes a self-complementary portion such that the overhang is capable of forming a hairpin structure that is stable under physiological conditions.

“Blunt” or “blunt end” means that there are no unpaired nucleotides at that end of the double stranded RNA agent, *i.e.*, no nucleotide overhang. A “blunt ended” double stranded RNA agent is double stranded over its entire length, *i.e.*, no nucleotide overhang at either end of the molecule. The

RNAi agents of the invention include RNAi agents with no nucleotide overhang at one end (*i.e.*, agents with one overhang and one blunt end) or with no nucleotide overhangs at either end. Most often such a molecule will be double-stranded over its entire length.

The term “antisense strand” or “guide strand” refers to the strand of an iRNA, *e.g.*, a dsRNA, which includes a region that is substantially complementary to a target sequence, *e.g.*, an APOC3 mRNA.

As used herein, the term “region of complementarity” refers to the region on the antisense strand that is substantially complementary to a sequence, for example a target sequence, *e.g.*, an apolipoprotein C3 nucleotide sequence, as defined herein. Where the region of complementarity is not fully complementary to the target sequence, the mismatches can be in the internal or terminal regions of the molecule. Generally, the most tolerated mismatches are in the terminal regions, *e.g.*, within 5, 4, or 3 nucleotides of the 5'- or 3'-end of the iRNA. In some embodiments, a double stranded RNA agent of the invention includes a nucleotide mismatch in the antisense strand. In some embodiments, the antisense strand of the double stranded RNA agent of the invention includes no more than 4 mismatches with the target mRNA, *e.g.*, the antisense strand includes 4, 3, 2, 1, or 0 mismatches with the target mRNA. In some embodiments, the antisense strand double stranded RNA agent of the invention includes no more than 4 mismatches with the sense strand, *e.g.*, the antisense strand includes 4, 3, 2, 1, or 0 mismatches with the sense strand. In some embodiments, a double stranded RNA agent of the invention includes a nucleotide mismatch in the sense strand. In some embodiments, the sense strand of the double stranded RNA agent of the invention includes no more than 4 mismatches with the antisense strand, *e.g.*, the sense strand includes 4, 3, 2, 1, or 0 mismatches with the antisense strand. In some embodiments, the nucleotide mismatch is, for example, within 5, 4, 3 nucleotides from the 3'-end of the iRNA. In another embodiment, the nucleotide mismatch is, for example, in the 3'-terminal nucleotide of the iRNA agent. In some embodiments, the mismatch(s) is not in the seed region.

Thus, an RNAi agent as described herein can contain one or more mismatches to the target sequence. In one embodiment, a RNAi agent as described herein contains no more than 3 mismatches (*i.e.*, 3, 2, 1, or 0 mismatches). In one embodiment, an RNAi agent as described herein contains no more than 2 mismatches. In one embodiment, an RNAi agent as described herein contains no more than 1 mismatch. In one embodiment, an RNAi agent as described herein contains 0 mismatches. In certain embodiments, if the antisense strand of the RNAi agent contains mismatches to the target sequence, the mismatch can optionally be restricted to be within the last 5 nucleotides from either the 5'- or 3'-end of the region of complementarity. For example, in such embodiments, for a 23 nucleotide RNAi agent, the strand which is complementary to a region of an APOC3 gene, generally does not contain any mismatch within the central 13 nucleotides. The methods described herein or methods known in the art can be used to determine whether an RNAi agent containing a mismatch to a target sequence is effective in inhibiting the expression of an APOC3 gene. Consideration of the efficacy of RNAi agents with mismatches in inhibiting expression of an APOC3 gene is important,

especially if the particular region of complementarity in an APOC3 gene is known to have polymorphic sequence variation within the population.

The term “sense strand” or “passenger strand” as used herein, refers to the strand of an iRNA that includes a region that is substantially complementary to a region of the antisense strand as that term is defined herein.

As used herein, “substantially all of the nucleotides are modified” are largely but not wholly modified and can include not more than 5, 4, 3, 2, or 1 unmodified nucleotides.

As used herein, the term “cleavage region” refers to a region that is located immediately adjacent to the cleavage site. The cleavage site is the site on the target at which cleavage occurs. In some embodiments, the cleavage region comprises three bases on either end of, and immediately adjacent to, the cleavage site. In some embodiments, the cleavage region comprises two bases on either end of, and immediately adjacent to, the cleavage site. In some embodiments, the cleavage site specifically occurs at the site bound by nucleotides 10 and 11 of the antisense strand, and the cleavage region comprises nucleotides 11, 12 and 13.

As used herein, and unless otherwise indicated, the term “complementary,” when used to describe a first nucleotide sequence in relation to a second nucleotide sequence, refers to the ability of an oligonucleotide or polynucleotide comprising the first nucleotide sequence to hybridize and form a duplex structure under certain conditions with an oligonucleotide or polynucleotide comprising the second nucleotide sequence, as will be understood by the skilled person. Such conditions can, for example, be stringent conditions, where stringent conditions can include: 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 50°C or 70°C for 12-16 hours followed by washing (see, *e.g.*, “Molecular Cloning: A Laboratory Manual, Sambrook, *et al.* (1989) Cold Spring Harbor Laboratory Press). Other conditions, such as physiologically relevant conditions as can be encountered inside an organism, can apply. The skilled person will be able to determine the set of conditions most appropriate for a test of complementarity of two sequences in accordance with the ultimate application of the hybridized nucleotides.

Complementary sequences within an iRNA, *e.g.*, within a dsRNA as described herein, include base-pairing of the oligonucleotide or polynucleotide comprising a first nucleotide sequence to an oligonucleotide or polynucleotide comprising a second nucleotide sequence over the entire length of one or both nucleotide sequences. Such sequences can be referred to as “fully complementary” with respect to each other herein. However, where a first sequence is referred to as “substantially complementary” with respect to a second sequence herein, the two sequences can be fully complementary, or they can form one or more, but generally not more than 5, 4, 3, or 2 mismatched base pairs upon hybridization for a duplex up to 30 base pairs, while retaining the ability to hybridize under the conditions most relevant to their ultimate application, *e.g.*, inhibition of gene expression *via* a RISC pathway. However, where two oligonucleotides are designed to form, upon hybridization, one or more single stranded overhangs, such overhangs shall not be regarded as mismatches with regard to the determination of complementarity. For example, a dsRNA comprising one oligonucleotide 21 nucleotides in length and another oligonucleotide 23 nucleotides in length, wherein

the longer oligonucleotide comprises a sequence of 21 nucleotides that is fully complementary to the shorter oligonucleotide, can yet be referred to as “fully complementary” for the purposes described herein.

“Complementary” sequences, as used herein, can also include, or be formed entirely from, non-Watson-Crick base pairs or base pairs formed from non-natural and modified nucleotides, in so far as the above requirements with respect to their ability to hybridize are fulfilled. Such non-Watson-Crick base pairs include, but are not limited to, G:U Wobble or Hoogsteen base pairing.

The terms “complementary,” “fully complementary” and “substantially complementary” herein can be used with respect to the base matching between the sense strand and the antisense strand of a dsRNA, or between the antisense strand of a double stranded RNA agent and a target sequence, as will be understood from the context of their use.

As used herein, a polynucleotide that is “substantially complementary to at least part of” a messenger RNA (mRNA) refers to a polynucleotide that is substantially complementary to a contiguous portion of the mRNA of interest (*e.g.*, an mRNA encoding an apolipoprotein C3 gene). For example, a polynucleotide is complementary to at least a part of an apolipoprotein C3 mRNA if the sequence is substantially complementary to a non-interrupted portion of an mRNA encoding an apolipoprotein C3 gene.

Accordingly, in some embodiments, the antisense polynucleotides disclosed herein are fully complementary to the target APOC3 sequence. In other embodiments, the antisense polynucleotides disclosed herein are substantially complementary to the target APOC3 sequence and comprise a contiguous nucleotide sequence which is at least 80% complementary over its entire length to the equivalent region of the nucleotide sequence of any one of SEQ ID NOs:1, 3, 5, 7, 9, or 11, or a fragment of any one of SEQ ID NOs:1-1, 3, 5, 7, 9, or 11, such as about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In some embodiments, the antisense polynucleotides disclosed herein are substantially complementary to a fragment of a target APOC3 sequence and comprise a contiguous nucleotide sequence which is at least 80% complementary over its entire length to a fragment of SEQ ID NO: 1 selected from the group of nucleotides; 232-254; 233-255; 238-260; 239-261; 242-264; 243-265; 244-266; 264-286; 268-290; 426-448; 431-453; 432-454; 433-455; 435-457; 436-458; 499-521; 500-522; 503-525; 504-526; 507-529; 510-532; and 511-533 of SEQ ID NO: 1, such as about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In other embodiments, the antisense polynucleotides disclosed herein are substantially complementary to a fragment of a target APOC3 sequence and comprise a contiguous nucleotide sequence which is at least 80% complementary over its entire length to a fragment of SEQ ID NO: 1 selected from the group of nucleotides 235-257; 238-260; 242-264; 243-265; 244-266; 426-448; 430-450; 431-453; 432-454; 433-455; 435-457; 436-458; 499-521; 503-525; and 504-526 of SEQ ID NO: 1, such as about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about

96%, about 97%, about 98%, or about 99% complementary. In some embodiments, the antisense polynucleotides disclosed herein are substantially complementary to a fragment of a target APOC3 sequence and comprise a contiguous nucleotide sequence which is at least 80% complementary over its entire length to a fragment of SEQ ID NO: 1 selected from the group of nucleotides 232-254; 239-261; 242-264; 244-266; 258-280; 264-286; 268-290; 429-451; 430-450; 430-452; 433-455; 434-456; 435-457; 500-522; 503-525; 507-529; and 510-532; and 504-526 of SEQ ID NO: 1, such as about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In other embodiments, the antisense polynucleotides disclosed herein are substantially complementary to a fragment of a target APOC3 sequence and comprise a contiguous nucleotide sequence which is at least 80% complementary over its entire length to a fragment of SEQ ID NO: 1 selected from the group of nucleotides 429-451; 430-452; 431-451; 432-452; 433-455; 504-526; and 506-526 of SEQ ID NO: 1, such as about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In other embodiments, the antisense polynucleotides disclosed herein are substantially complementary to the target APOC3 sequence and comprise a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to any one of the sense strand nucleotide sequences in any one of any one of Tables 2-5, 14, and 15, or a fragment of any one of the sense strand nucleotide sequences in any one of Tables 2-5, 14, and 15, such as about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or 100% complementary.

In one embodiment, an RNAi agent of the disclosure includes a sense strand that is substantially complementary to an antisense polynucleotide which, in turn, is the same as a target APOC3 sequence, and wherein the sense strand polynucleotide comprises a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to the equivalent region of the nucleotide sequence of SEQ ID NOs: 2, 4, 6, 8, 10, or 12, or a fragment of any one of SEQ ID NOs: 2, 4, 6, 8, 10, or 12, such as about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or 100% complementary.

In some embodiments, an iRNA of the invention includes a sense strand that is substantially complementary to an antisense polynucleotide which, in turn, is complementary to a target apolipoprotein C3 sequence, and wherein the sense strand polynucleotide comprises a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to any one of the antisense strand nucleotide sequences in any one of any one of Tables 2-5, 14, and 15, or a fragment of any one of the antisense strand nucleotide sequences in any one of Tables 2-5, 14, and 15, such as about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or 100% complementary.

In certain embodiments, the sense and antisense strands are selected from any one of duplexes AD-959917.1; AD-959918.1; AD-960096.1; AD-960064.1; AD-959914.1; AD-959941.1; AD-960031.1; AD-960063.1; AD-960293.1; AD-960288.1; AD-960445.1; AD-960292.1; AD-960475.1;

AD-960442.1; AD-960470.1; AD-960436.1; AD-960446.1; AD-960474.1; AD-960294.1; AD-960471.1; AD-960314.1; AD-960443.1; AD-960282.1; AD-960283.1; AD-80794.7; AD-960478.1; AD-960481.1; or AD-960482.1.

In other embodiments, the sense and antisense strands are selected from any one of duplexes
 5 AD-959917.1; AD-960064.1; AD-960031.1; AD-960063.1; AD-960293.1; AD-960288.1; AD-960445.1; AD-960292.1; AD-960475.1; AD-960442.1; AD-960470.1; AD-960436.1; AD-960446.1; AD-960474.1; AD-960294.1; AD-960443.1; AD-80794.7; and AD-959910.1.

In some embodiments, the sense and antisense strands are selected from any one of duplexes
 AD-80794.8; AD-959907.2; AD-959914.2; AD-959916.2; AD-959932.2; AD-960314.2; AD-
 10 959941.2; AD-960030.2; AD-960062.2; AD-960064.2; AD-960065.2; AD-960066.2; AD-960294.2; AD-960471.2; AD-960474.2; AD-960478.2; and AD-960481.2.

In other embodiments, the sense and antisense strands are selected from any one of duplexes
 AD-960030; AD-1143243; AD-1143245; AD-1143247; AD-1143249; AD-1143256; AD-1143260;
 AD-1143278; AD-1143287; AD-1143295; AD-1143299; AD-1143302; and AD-1143305.

15 In one embodiment, the sense and antisense strands are of duplex AD-1143243.

In general, an “iRNA” includes ribonucleotides with chemical modifications. Such modifications may include all types of modifications disclosed herein or known in the art. Any such modifications, as used in a dsRNA molecule, are encompassed by “iRNA” for the purposes of this specification and claims.

20 In certain embodiments of the instant disclosure, inclusion of a deoxy-nucleotide if present within an RNAi agent can be considered to constitute a modified nucleotide.

In an aspect of the invention, an agent for use in the methods and compositions of the invention is a single-stranded antisense oligonucleotide molecule that inhibits a target mRNA *via* an antisense inhibition mechanism. The single-stranded antisense oligonucleotide molecule is
 25 complementary to a sequence within the target mRNA. The single-stranded antisense oligonucleotides can inhibit translation in a stoichiometric manner by base pairing to the mRNA and physically obstructing the translation machinery, see Dias, N. *et al.*, (2002) *Mol Cancer Ther* 1:347-355. The single-stranded antisense oligonucleotide molecule may be about 14 to about 30 nucleotides in length and have a sequence that is complementary to a target sequence. For example, the single-
 30 stranded antisense oligonucleotide molecule may comprise a sequence that is at least about 14, 15, 16, 17, 18, 19, 20, or more contiguous nucleotides from any one of the antisense sequences described herein.

The phrase “contacting a cell with an iRNA,” such as a dsRNA, as used herein, includes contacting a cell by any possible means. Contacting a cell with an iRNA includes contacting a cell *in vitro* with the iRNA or contacting a cell *in vivo* with the iRNA. The contacting may be done directly or indirectly. Thus, for example, the iRNA may be put into physical contact with the cell by the
 35 individual performing the method, or alternatively, the iRNA may be put into a situation that will permit or cause it to subsequently come into contact with the cell.

Contacting a cell *in vitro* may be done, for example, by incubating the cell with the iRNA. Contacting a cell *in vivo* may be done, for example, by injecting the iRNA into or near the tissue where the cell is located, or by injecting the iRNA into another area, *e.g.*, the bloodstream or the subcutaneous space, such that the agent will subsequently reach the tissue where the cell to be contacted is located. For example, the iRNA may contain or be coupled to a ligand, *e.g.*, GalNAc, that directs the iRNA to a site of interest, *e.g.*, the liver. Combinations of *in vitro* and *in vivo* methods of contacting are also possible. For example, a cell may also be contacted *in vitro* with an iRNA and subsequently transplanted into a subject.

In certain embodiments, contacting a cell with an iRNA includes “introducing” or “delivering the iRNA into the cell” by facilitating or effecting uptake or absorption into the cell. Absorption or uptake of an iRNA can occur through unaided diffusion or active cellular processes, or by auxiliary agents or devices. Introducing an iRNA into a cell may be *in vitro* or *in vivo*. For example, for *in vivo* introduction, iRNA can be injected into a tissue site or administered systemically. *In vitro* introduction into a cell includes methods known in the art such as electroporation and lipofection. Further approaches are described herein below or are known in the art.

The term “lipid nanoparticle” or “LNP” is a vesicle comprising a lipid layer encapsulating a pharmaceutically active molecule, such as a nucleic acid molecule, *e.g.*, an iRNA or a plasmid from which an iRNA is transcribed. LNPs are described in, for example, U.S. Patent Nos. 6,858,225, 6,815,432, 8,158,601, and 8,058,069, the entire contents of which are hereby incorporated herein by reference.

As used herein, a “subject” is an animal, such as a mammal, including a primate (such as a human, a non-human primate, *e.g.*, a monkey, and a chimpanzee), a non-primate (such as a cow, a pig, a horse, a goat, a rabbit, a sheep, a hamster, a guinea pig, a cat, a dog, a rat, or a mouse), or a bird that expresses the target gene, either endogenously or heterologously. In an embodiment, the subject is a human, such as a human being treated or assessed for a disease or disorder that would benefit from reduction in APOC3 expression; a human at risk for a disease or disorder that would benefit from reduction in APOC3 expression; a human having a disease or disorder that would benefit from reduction in APOC3 expression; or human being treated for a disease or disorder that would benefit from reduction in APOC3 expression as described herein. In some embodiments, the subject is a female human. In other embodiments, the subject is a male human. In one embodiment, the subject is an adult subject. In another embodiment, the subject is a pediatric subject.

As used herein, the terms “treating” or “treatment” refer to a beneficial or desired result, such as reducing at least one sign or symptom of an APOC3-associated disorder in a subject. Treatment also includes a reduction of one or more sign or symptoms associated with unwanted APOC3 expression; diminishing the extent of unwanted APOC3 activation or stabilization; amelioration or palliation of unwanted APOC3 activation or stabilization. “Treatment” can also mean prolonging survival as compared to expected survival in the absence of treatment. The term “lower” in the context of the level of APOC3 in a subject or a disease marker or symptom refers to a statistically significant decrease in such level. The decrease can be, for example, at least 10%, 15%, 20%, 25%, 30%, %, ,

40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more. In certain embodiments, a decrease is at least 20%. In certain embodiments, the decrease is at least 50% in a disease marker, *e.g.*, protein or gene expression level. “Lower” in the context of the level of APOC3 in a subject is preferably down to a level accepted as within the range of normal for an individual without such disorder. In certain embodiments, “lower” is the decrease in the difference between the level of a marker or symptom for a subject suffering from a disease and a level accepted within the range of normal for an individual, *e.g.*, the level of decrease in bodyweight between an obese individual and an individual having a weight accepted within the range of normal.

As used herein, “prevention” or “preventing,” when used in reference to a disease, disorder or condition thereof, may be treated or ameliorated by a reduction in expression of an APOC3 gene, refers to a reduction in the likelihood that a subject will develop a symptom associated with such a disease, disorder, or condition, *e.g.*, a symptom of unwanted or excessive APOC3 expression, such as hypertriglyceridemia. The likelihood of developing, *e.g.*, hypertriglyceridemia, is reduced, for example, when an individual having one or more risk factors for hypertriglyceridemia either fails to develop hypertriglyceridemia or develops hypertriglyceridemia with less severity relative to a population having the same risk factors and not receiving treatment as described herein. The failure to develop a disease, disorder or condition, or the reduction in the development of a symptom associated with such a disease, disorder or condition (*e.g.*, by at least about 10% on a clinically accepted scale for that disease or disorder), or the exhibition of delayed symptoms delayed (*e.g.*, by days, weeks, months or years) is considered effective prevention.

As used herein, the term “apolipoprotein C3-associated disease” or “APOC3-associated disease,” is a disease, disorder or a condition that is caused by, or is associated with, unwanted or excessive APOC3 expression. The term “APOC3-associated disease” includes a disease, disorder or condition that may be treated or ameliorated by a reduction in APOC3 expression. The term APOC3-associated disease” includes hypertriglyceridemia, or a high triglyceride levels.

The levels of triglycerides in a serum of a subject, *e.g.*, a human subject, that may be indicative of hypertriglyceridemia are described in Oh, R. C. *et al.*, (2007) *American Family Physician*, 75(9):1366-1371. Specifically, hypertriglyceridemia may be associated with “borderline-high serum triglyceride levels” (*i.e.*, 150 to 199 mg per dL or 1.70 to 2.25 mmol per L); “high serum triglyceride levels” (*i.e.*, 200 to 499 mg per dL or 2.26 to 5.64 mmol per L); or “very high triglyceride levels” (*i.e.*, 500 mg per dL or higher (or 5.65 mmol per L or higher)

In one embodiment, an APOC3-associated disease is primary hypertriglyceridemia. “Primary triglyceridemia” results from environmental or genetic causes (*e.g.*, a result of no obvious underlying medical cause). Exemplary diseases characterized as primary hypertriglyceridemias include, but are not limited to, familial chylomicronemia (hyperlipoproteinemia type I), primary mixed hyperlipidemia (type 5), familial hypertriglyceridemia (hyperlipoproteinemia type 4), familial combined hyperlipoproteinemia (type 2B) and familial dysbetalipoproteinemia (hyperlipoproteinemia type 3).

In another embodiment, an APOC3-associated disease is secondary hypertriglyceridemia. "Secondary triglyceridemia" is caused by, or be associated with, other underlying disorders and conditions. Such disorders and/or conditions include, *e.g.*, obesity, metabolic syndrome, diabetes, fatty liver, alcohol use, renal disease, pregnancy, nonalcoholic fatty liver disorder, hypothyroidism, paraproteinemias (such as hypergammaglobulinemia in macroglobulinemia, myeloma, lymphoma and lymphocytic leukemias), autoimmune disorders (such as systemic lupus erythematosus), intake of medications (such as antiretroviral drugs, including ritonavir and lopinavir, and antipsychotic medications, including clozapine and olanzapine), see G. Yuan *et al.*, (2007) *Canadian Medical Association Journal*, 176(8):1113-1120.

Any disorder that may be a cause of hypertriglyceridemia (*e.g.*, secondary hypertriglyceridemia) or that may be a consequence of hypertriglyceridemia (*e.g.*, primary or secondary hypertriglyceridemia) is encompassed by the term "APOC3-associated disease". Non-limiting examples of APOC3-associated diseases include metabolic disorders, *e.g.*, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, polycystic ovary syndrome, kidney disease, obesity, type 2 diabetes mellitus (insulin resistance); hypertension; cardiovascular disorders, *e.g.*, atherosclerosis; and pancreatitis, *e.g.*, acute pancreatitis.

"Therapeutically effective amount," as used herein, is intended to include the amount of an RNAi agent that, when administered to a subject having an APOC3-associated disease, is sufficient to effect treatment of the disease (*e.g.*, by diminishing, ameliorating, or maintaining the existing disease or one or more symptoms of disease). The "therapeutically effective amount" may vary depending on the RNAi agent, how the agent is administered, the disease and its severity and the history, age, weight, family history, genetic makeup, the types of preceding or concomitant treatments, if any, and other individual characteristics of the subject to be treated.

"Prophylactically effective amount," as used herein, is intended to include the amount of an RNAi agent that, when administered to a subject having an APOC3-associated disorder, is sufficient to prevent or ameliorate the disease or one or more symptoms of the disease. Ameliorating the disease includes slowing the course of the disease or reducing the severity of later-developing disease. The "prophylactically effective amount" may vary depending on the RNAi agent, how the agent is administered, the degree of risk of disease, and the history, age, weight, family history, genetic makeup, the types of preceding or concomitant treatments, if any, and other individual characteristics of the patient to be treated.

A "therapeutically-effective amount" or "prophylactically effective amount" also includes an amount of an RNAi agent that produces some desired effect at a reasonable benefit/risk ratio applicable to any treatment. The iRNA employed in the methods of the present invention may be administered in a sufficient amount to produce a reasonable benefit/risk ratio applicable to such treatment.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human subjects and animal subjects without excessive

toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically-acceptable carrier" as used herein means a pharmaceutically-acceptable material, composition, or vehicle, such as a liquid or solid filler, diluent, excipient, manufacturing aid (*e.g.*, lubricant, talc magnesium, calcium or zinc stearate, or steric acid), or solvent encapsulating material, involved in carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the subject being treated. Such carriers are known in the art. Pharmaceutically acceptable carriers include carriers for administration by injection.

The term "sample," as used herein, includes a collection of similar fluids, cells, or tissues isolated from a subject, as well as fluids, cells, or tissues present within a subject. Examples of biological fluids include blood, serum and serosal fluids, plasma, cerebrospinal fluid, ocular fluids, lymph, urine, saliva, and the like. Tissue samples may include samples from tissues, organs, or localized regions. For example, samples may be derived from particular organs, parts of organs, or fluids or cells within those organs. In certain embodiments, samples may be derived from the liver (*e.g.*, whole liver or certain segments of liver or certain types of cells in the liver, such as, *e.g.*, hepatocytes). In some embodiments, a "sample derived from a subject" refers to urine obtained from the subject. A "sample derived from a subject" can refer to blood or blood derived serum or plasma from the subject.

II. iRNAs of the Invention

The present invention provides iRNAs which inhibit the expression of an apolipoprotein C3 gene. In preferred embodiments, the iRNA includes double stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of an APOC3 gene in a cell, such as a cell within a subject, *e.g.*, a mammal, such as a human susceptible to developing an apolipoprotein C3-associated disorder, *e.g.*, hypertriglyceridemia. The dsRNAi agent includes an antisense strand having a region of complementarity which is complementary to at least a part of an mRNA formed in the expression of an APOC3 gene. The region of complementarity is about 19-30 nucleotides in length (*e.g.*, about 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, or 19 nucleotides in length). Upon contact with a cell expressing the APOC3 gene, the iRNA inhibits the expression of the APOC3 gene (*e.g.*, a human, a primate, a non-primate, or a rat APOC3 gene) by at least about 50% as assayed by, for example, a PCR or branched DNA (bDNA)-based method, or by a protein-based method, such as by immunofluorescence analysis, using, for example, western blotting or flow cytometric techniques. In preferred embodiments, inhibition of expression is determined by the qPCR method provided in the examples herein with the siRNA at, *e.g.*, a 10 nM concentration, in an appropriate organism cell line provided therein. In preferred embodiments, inhibition of expression *in vivo* is determined by knockdown of the human gene in a rodent expressing the human gene, *e.g.*, a mouse or an AAV-

infected mouse expressing the human target gene, *e.g.*, when administered as single dose, *e.g.*, at 3 mg/kg at the nadir of RNA expression.

A dsRNA includes two RNA strands that are complementary and hybridize to form a duplex structure under conditions in which the dsRNA will be used. One strand of a dsRNA (the antisense strand) includes a region of complementarity that is substantially complementary, and generally fully complementary, to a target sequence. The target sequence can be derived from the sequence of an mRNA formed during the expression of an APOC3 gene. The other strand (the sense strand) includes a region that is complementary to the antisense strand, such that the two strands hybridize and form a duplex structure when combined under suitable conditions. As described elsewhere herein and as known in the art, the complementary sequences of a dsRNA can also be contained as self-complementary regions of a single nucleic acid molecule, as opposed to being on separate oligonucleotides.

Generally, the duplex structure is 15 to 30 base pairs in length, *e.g.*, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs in length. In certain preferred embodiments, the duplex structure is 18 to 25 base pairs in length, *e.g.*, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-25, 20-24, 20-23, 20-22, 20-21, 21-25, 21-24, 21-23, 21-22, 22-25, 22-24, 22-23, 23-25, 23-24 or 24-25 base pairs in length, for example, 19-21 basepairs in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the disclosure.

Similarly, the region of complementarity to the target sequence is 15 to 30 nucleotides in length, *e.g.*, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length, for example 19-23 nucleotides in length or 21-23 nucleotides in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the disclosure.

In some embodiments, the duplex structure is 19 to 30 base pairs in length. Similarly, the region of complementarity to the target sequence is 19 to 30 nucleotides in length.

In some embodiments, the dsRNA is about 19 to about 23 nucleotides in length, or about 25 to about 30 nucleotides in length. In general, the dsRNA is long enough to serve as a substrate for the Dicer enzyme. For example, it is well-known in the art that dsRNAs longer than about 21-23 nucleotides in length may serve as substrates for Dicer. As the ordinarily skilled person will also recognize, the region of an RNA targeted for cleavage will most often be part of a larger RNA molecule, often an mRNA molecule. Where relevant, a “part” of an mRNA target is a contiguous sequence of an mRNA target of sufficient length to allow it to be a substrate for RNAi-directed cleavage (*i.e.*, cleavage through a RISC pathway).

One of skill in the art will also recognize that the duplex region is a primary functional portion of a dsRNA, *e.g.*, a duplex region of about 19 to about 30 base pairs, *e.g.*, about 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs. Thus, in one embodiment, to the extent that it becomes processed to a functional duplex, of *e.g.*, 15-30 base pairs, that targets a desired RNA for cleavage, an RNA molecule or complex of RNA molecules having a duplex region greater than 30 base pairs is a dsRNA. Thus, an ordinarily skilled artisan will recognize that in one embodiment, a miRNA is a dsRNA. In another embodiment, a dsRNA is not a naturally occurring miRNA. In another embodiment, an iRNA agent useful to target apolipoprotein C3 gene expression is not generated in the target cell by cleavage of a larger dsRNA.

A dsRNA as described herein can further include one or more single-stranded nucleotide overhangs *e.g.*, 1-4, 2-4, 1-3, 2-3, 1, 2, 3, or 4 nucleotides. dsRNAs having at least one nucleotide overhang can have superior inhibitory properties relative to their blunt-ended counterparts. A nucleotide overhang can comprise or consist of a nucleotide/nucleoside analog, including a deoxynucleotide/nucleoside. The overhang(s) can be on the sense strand, the antisense strand, or any combination thereof. Furthermore, the nucleotide(s) of an overhang can be present on the 5'-end, 3'-end, or both ends of an antisense or sense strand of a dsRNA.

A dsRNA can be synthesized by standard methods known in the art. Double stranded RNAi compounds of the invention may be prepared using a two-step procedure. First, the individual strands of the double stranded RNA molecule are prepared separately. Then, the component strands are annealed. The individual strands of the siRNA compound can be prepared using solution-phase or solid-phase organic synthesis or both. Organic synthesis offers the advantage that the oligonucleotide strands comprising unnatural or modified nucleotides can be easily prepared. Similarly, single-stranded oligonucleotides of the invention can be prepared using solution-phase or solid-phase organic synthesis or both.

In an aspect, a dsRNA of the invention includes at least two nucleotide sequences, a sense sequence and an anti-sense sequence. The sense strand is selected from the group of sequences provided in any one of Tables 2-5, 14, and 15, and the corresponding antisense strand of the sense strand is selected from the group of sequences of any one of Tables 2-5, 14, and 15. In this aspect, one of the two sequences is complementary to the other of the two sequences, with one of the sequences being substantially complementary to a sequence of an mRNA generated in the expression of an apolipoprotein C3 gene. As such, in this aspect, a dsRNA will include two oligonucleotides, where one oligonucleotide is described as the sense strand in any one of Tables 2-5, 14, and 15, and the second oligonucleotide is described as the corresponding antisense strand of the sense strand in any one of Tables 2-5, 14, and 15.

In certain embodiments, the substantially complementary sequences of the dsRNA are contained on separate oligonucleotides. In other embodiments, the substantially complementary sequences of the dsRNA are contained on a single oligonucleotide.

In certain embodiments, the sense or antisense strand is selected from the sense or antisense strand of any one of duplexes AD-959917.1; AD-959918.1; AD-960096.1; AD-960064.1; AD-959914.1; AD-959941.1; AD-960031.1; AD-960063.1; AD-960293.1; AD-960288.1; AD-960445.1; AD-960292.1; AD-960475.1; AD-960442.1; AD-960470.1; AD-960436.1; AD-960446.1; AD-960474.1; AD-960294.1; AD-960471.1; AD-960314.1; AD-960443.1; AD-960282.1; AD-960283.1; AD-80794.7; AD-960478.1; AD-960481.1; or AD-960482.1.

In other embodiments, the sense or antisense strand is selected from the sense or antisense strand of any one of duplexes AD-959917.1; AD-960064.1; AD-960031.1; AD-960063.1; AD-960293.1; AD-960288.1; AD-960445.1; AD-960292.1; AD-960475.1; AD-960442.1; AD-960470.1; AD-960436.1; AD-960446.1; AD-960474.1; AD-960294.1; AD-960443.1; AD-80794.7; and AD-959910.1.

In some embodiments, the sense or antisense strand is selected from the sense or antisense strand of any one of duplexes AD-80794.8; AD-959907.2; AD-959914.2; AD-959916.2; AD-959932.2; AD-960314.2; AD-959941.2; AD-960030.2; AD-960062.2; AD-960064.2; AD-960065.2; AD-960066.2; AD-960294.2; AD-960471.2; AD-960474.2; AD-960478.2; and AD-960481.2.

In some embodiments, the sense or antisense strand is selected from the sense or antisense strand of any one of duplexes AD-960030; AD-1143243; AD-1143245; AD-1143247; AD-1143249; AD-1143256; AD-1143260; AD-1143278; AD-1143287; AD-1143295; AD-1143299; AD-1143302; and AD-1143305.

In some embodiments, the sense or antisense strand is selected from the sense or antisense strand of duplex AD-1143243.

It will be understood that, although the sequences in Tables 2, 4, and 14 are not described as modified or conjugated sequences, the RNA of the iRNA of the invention *e.g.*, a dsRNA of the invention, may comprise any one of the sequences set forth in any one of Tables 2-5, 14, and 15 that is un-modified, un-conjugated, or modified or conjugated differently than described therein. In other words, the invention encompasses dsRNA of Tables 2-5, 14, and 15 which are un-modified, un-conjugated, modified, or conjugated, as described herein.

The skilled person is well aware that dsRNAs having a duplex structure of about 20 to 23 base pairs, *e.g.*, 21, base pairs have been hailed as particularly effective in inducing RNA interference (Elbashir *et al.*, *EMBO* 2001, 20:6877-6888). However, others have found that shorter or longer RNA duplex structures can also be effective (Chu and Rana (2007) *RNA* 14:1714-1719; Kim *et al.* (2005) *Nat Biotech* 23:222-226). In the embodiments described above, by virtue of the nature of the oligonucleotide sequences provided in any one of Tables 2-5, 14, and 15, dsRNAs described herein can include at least one strand of a length of minimally 21 nucleotides. It can be reasonably expected that shorter duplexes having any one of the sequences in any one of Tables 2-5, 14, and 15 minus only a few nucleotides on one or both ends can be similarly effective as compared to the dsRNAs described above. Hence, dsRNAs having a sequence of at least 19, 20, or more contiguous nucleotides derived from any one of the sequences of any one of Tables 2-5, 14, and 15, and differing in their ability to inhibit the expression of an apolipoprotein C3 gene by not more than about 5, 10, 15, 20, 25, or 30 %

inhibition from a dsRNA comprising the full sequence, are contemplated to be within the scope of the present invention.

In addition, the RNAs provided in Tables 2-5, 14, and 15 identify a site(s) in an apolipoprotein C3 transcript that is susceptible to RISC-mediated cleavage. As such, the present invention further features iRNAs that target within one of these sites. As used herein, an iRNA is said to target within a particular site of an RNA transcript if the iRNA promotes cleavage of the transcript anywhere within that particular site. Such an iRNA will generally include at least about 19 contiguous nucleotides from any one of the sequences provided in any one of Tables 2-5, 14, and 15 coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in an apolipoprotein C3 gene.

III. Modified iRNAs of the Invention

In certain embodiments, the RNA of the iRNA of the invention *e.g.*, a dsRNA, is unmodified, and does not comprise, *e.g.*, chemical modifications or conjugations known in the art and described herein. In other embodiments, the RNA of an iRNA of the invention, *e.g.*, a dsRNA, is chemically modified to enhance stability or other beneficial characteristics. In certain embodiments of the invention, substantially all of the nucleotides of an iRNA of the invention are modified. In other embodiments of the invention, all of the nucleotides of an iRNA or substantially all of the nucleotides of an iRNA are modified, *i.e.*, not more than 5, 4, 3, 2, or 1 unmodified nucleotides are present in a strand of the iRNA.

The nucleic acids featured in the invention can be synthesized or modified by methods well established in the art, such as those described in "Current protocols in nucleic acid chemistry," Beaucage, S.L. *et al.* (Edrs.), John Wiley & Sons, Inc., New York, NY, USA, which is hereby incorporated herein by reference. Modifications include, for example, end modifications, *e.g.*, 5'-end modifications (phosphorylation, conjugation, inverted linkages) or 3'-end modifications (conjugation, DNA nucleotides, inverted linkages, *etc.*); base modifications, *e.g.*, replacement with stabilizing bases, destabilizing bases, or bases that base pair with an expanded repertoire of partners, removal of bases (abasic nucleotides), or conjugated bases; sugar modifications (*e.g.*, at the 2'-position or 4'-position) or replacement of the sugar; or backbone modifications, including modification or replacement of the phosphodiester linkages. Specific examples of iRNA compounds useful in the embodiments described herein include, but are not limited to RNAs containing modified backbones or no natural internucleoside linkages. RNAs having modified backbones include, among others, those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, modified RNAs that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides. In some embodiments, a modified iRNA will have a phosphorus atom in its internucleoside backbone.

Modified RNA backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates,

phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5'-linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'.

- 5 Various salts, mixed salts and free acid forms are also included. In some embodiments of the invention, the dsRNA agents of the invention are in a free acid form. In other embodiments of the invention, the dsRNA agents of the invention are in a salt form. In one embodiment, the dsRNA agents of the invention are in a sodium salt form. In certain embodiments, when the dsRNA agents of the invention are in the sodium salt form, sodium ions are present in the agent as counterions for
- 10 substantially all of the phosphodiester and/or phosphorothioate groups present in the agent. Agents in which substantially all of the phosphodiester and/or phosphorothioate linkages have a sodium counterion include not more than 5, 4, 3, 2, or 1 phosphodiester and/or phosphorothioate linkages without a sodium counterion. In some embodiments, when the dsRNA agents of the invention are in the sodium salt form, sodium ions are present in the agent as counterions for all of the phosphodiester and/or phosphorothioate groups present in the agent.
- 15

Representative U.S. Patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S. Patent Nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,195; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,316; 5,550,111; 5,563,253; 20 5,571,799; 5,587,361; 5,625,050; 6,028,188; 6,124,445; 6,160,109; 6,169,170; 6,172,209; 6,239,265; 6,277,603; 6,326,199; 6,346,614; 6,444,423; 6,531,590; 6,534,639; 6,608,035; 6,683,167; 6,858,715; 6,867,294; 6,878,805; 7,015,315; 7,041,816; 7,273,933; 7,321,029; and U.S. Pat RE39464, the entire contents of each of which are hereby incorporated herein by reference.

- Modified RNA backbones that do not include a phosphorus atom therein have backbones that
- 25 are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatoms and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones;
- 30 alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S, and CH₂ component parts.

Representative U.S. Patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S. Patent Nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 35 5,235,033; 5,64,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439, the entire contents of each of which are hereby incorporated herein by reference.

Suitable RNA mimetics are contemplated for use in iRNAs provided herein, in which both the sugar and the internucleoside linkage, *i.e.*, the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound in which an RNA mimetic that has been shown to have excellent hybridization properties is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar backbone of an RNA is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative US patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Patent Nos. 5,539,082; 5,714,331; and 5,719,262, the entire contents of each of which are hereby incorporated herein by reference. Additional PNA compounds suitable for use in the iRNAs of the invention are described in, for example, in Nielsen *et al.*, *Science*, 1991, 254, 1497-1500.

Some embodiments featured in the invention include RNAs with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular --CH₂--NH--CH₂--, --CH₂--N(CH₃)--O--CH₂--[known as a methylene (methylimino) or MMI backbone], --CH₂--O--N(CH₃)--CH₂--, --CH₂--N(CH₃)--N(CH₃)--CH₂-- and --N(CH₃)--CH₂--CH₂--[wherein the native phosphodiester backbone is represented as --O--P--O--CH₂--] of the above-referenced U.S. Patent No. 5,489,677, and the amide backbones of the above-referenced U.S. Patent No. 5,602,240. In some embodiments, the RNAs featured herein have morpholino backbone structures of the above-referenced U.S. Patent No. 5,034,506.

Modified RNAs can also contain one or more substituted sugar moieties. The iRNAs, *e.g.*, dsRNAs, featured herein can include one of the following at the 2'-position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl can be substituted or unsubstituted C₁ to C₁₀ alkyl or C₂ to C₁₀ alkenyl and alkynyl. Exemplary suitable modifications include O[(CH₂)_nO]_mCH₃, O(CH₂)_nOCH₃, O(CH₂)_nNH₂, O(CH₂)_nCH₃, O(CH₂)_nONH₂, and O(CH₂)_nON[(CH₂)_nCH₃]₂, where n and m are from 1 to about 10. In other embodiments, dsRNAs include one of the following at the 2' position: C₁ to C₁₀ lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH₃, OCN, Cl, Br, CN, CF₃, OCF₃, SOCH₃, SO₂CH₃, ONO₂, NO₂, N₃, NH₂, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an iRNA, or a group for improving the pharmacodynamic properties of an iRNA, and other substituents having similar properties. In some embodiments, the modification includes a 2'-methoxyethoxy (2'-O--CH₂CH₂OCH₃, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin *et al.*, *Helv. Chim. Acta*, 1995, 78:486-504) *i.e.*, an alkoxy-alkoxy group. Another exemplary modification is 2'-dimethylaminoethoxy, *i.e.*, a O(CH₂)₂ON(CH₃)₂ group, also known as 2'-DMAOE, as described in examples herein below, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), *i.e.*, 2'-O--CH₂--O--CH₂--N(CH₂)₂. Further exemplary modifications include : 5'-Me-2'-F nucleotides, 5'-Me-2'-OMe nucleotides, 5'-Me-2'-

deoxynucleotides, (both R and S isomers in these three families); 2'-alkoxyalkyl; and 2'-NMA (N-methylacetamide).

Other modifications include 2'-methoxy (2'-OCH₃), 2'-aminopropoxy (2'-OCH₂CH₂CH₂NH₂) and 2'-fluoro (2'-F). Similar modifications can also be made at other positions on the RNA of an
5 iRNA, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked dsRNAs and the 5' position of 5' terminal nucleotide. iRNAs can also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative US patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S. Patent Nos. 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427;
10 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, certain of which are commonly owned with the instant application,. The entire contents of each of the foregoing are hereby incorporated herein by reference.

An iRNA can also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the
15 purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C), and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as deoxy-thymine (dT), 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and
20 cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo, particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-daazaadenine and 3-deazaguanine and 3-deazaadenine. Further nucleobases include those
25 disclosed in U.S. Pat. No. 3,687,808, those disclosed in Modified Nucleosides in Biochemistry, Biotechnology and Medicine, Herdewijn, P. ed. Wiley-VCH, 2008; those disclosed in The Concise Encyclopedia Of Polymer Science And Engineering, pages 858-859, Kroschwitz, J. L, ed. John Wiley & Sons, 1990, these disclosed by Englisch *et al.*, Angewandte Chemie, International Edition, 1991, 30, 613, and those disclosed by Sanghvi, Y S., Chapter 15, dsRNA Research and Applications, pages
30 289-302, Crooke, S. T. and Lebleu, B., Ed., CRC Press, 1993. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds featured in the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C
35 (Sanghvi, Y. S., Crooke, S. T. and Lebleu, B., Eds., dsRNA Research and Applications, CRC Press, Boca Raton, 1993, pp. 276-278) and are exemplary base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

Representative U.S. Patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include, but are not limited to, the above noted

U.S. Patent Nos. 3,687,808, 4,845,205; 5,130,30; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; 5,681,941; 5,750,692; 6,015,886; 6,147,200; 6,166,197; 6,222,025; 6,235,887; 6,380,368; 6,528,640; 6,639,062; 6,617,438; 7,045,610; 7,427,672; and 7,495,088, the entire contents of each of which are hereby incorporated herein by reference.

The RNA of an iRNA can also be modified to include one or more locked nucleic acids (LNA). A locked nucleic acid is a nucleotide having a modified ribose moiety in which the ribose moiety comprises an extra bridge connecting the 2' and 4' carbons. This structure effectively "locks" the ribose in the 3'-endo structural conformation. The addition of locked nucleic acids to siRNAs has been shown to increase siRNA stability in serum, and to reduce off-target effects (Elmen, J. *et al.*, (2005) *Nucleic Acids Research* 33(1):439-447; Mook, OR. *et al.*, (2007) *Mol Canc Ther* 6(3):833-843; Grunweller, A. *et al.*, (2003) *Nucleic Acids Research* 31(12):3185-3193).

In some embodiments, the RNA of an iRNA can also be modified to include one or more bicyclic sugar moieties. A "bicyclic sugar" is a furanosyl ring modified by the bridging of two atoms. A "bicyclic nucleoside" ("BNA") is a nucleoside having a sugar moiety comprising a bridge connecting two carbon atoms of the sugar ring, thereby forming a bicyclic ring system. In certain embodiments, the bridge connects the 4'-carbon and the 2'-carbon of the sugar ring. Thus, in some embodiments an agent of the invention may include one or more locked nucleic acids (LNA). A locked nucleic acid is a nucleotide having a modified ribose moiety in which the ribose moiety comprises an extra bridge connecting the 2' and 4' carbons. In other words, an LNA is a nucleotide comprising a bicyclic sugar moiety comprising a 4'-CH₂-O-2' bridge. This structure effectively "locks" the ribose in the 3'-endo structural conformation. The addition of locked nucleic acids to siRNAs has been shown to increase siRNA stability in serum, and to reduce off-target effects (Elmen, J. *et al.*, (2005) *Nucleic Acids Research* 33(1):439-447; Mook, OR. *et al.*, (2007) *Mol Canc Ther* 6(3):833-843; Grunweller, A. *et al.*, (2003) *Nucleic Acids Research* 31(12):3185-3193). Examples of bicyclic nucleosides for use in the polynucleotides of the invention include without limitation nucleosides comprising a bridge between the 4' and the 2' ribosyl ring atoms. In certain embodiments, the antisense polynucleotide agents of the invention include one or more bicyclic nucleosides comprising a 4' to 2' bridge. Examples of such 4' to 2' bridged bicyclic nucleosides, include but are not limited to 4'-(CH₂)—O-2' (LNA); 4'-(CH₂)—S-2'; 4'-(CH₂)₂—O-2' (ENA); 4'-CH(CH₃)—O-2' (also referred to as "constrained ethyl" or "cEt") and 4'-CH(CH₂OCH₃)—O-2' (and analogs thereof; see, *e.g.*, U.S. Patent No. 7,399,845); 4'-C(CH₃)(CH₃)—O-2' (and analogs thereof; see *e.g.*, U.S. Patent No. 8,278,283); 4'-CH₂—N(OCH₃)-2' (and analogs thereof; see *e.g.*, U.S. Patent No. 8,278,425); 4'-CH₂—O—N(CH₃)-2' (see, *e.g.*, U.S. Patent Publication No. 2004/0171570); 4'-CH₂—N(R)—O-2', wherein R is H, C1-C12 alkyl, or a protecting group (see, *e.g.*, U.S. Patent No. 7,427,672); 4'-CH₂—C(H)(CH₃)-2' (see, *e.g.*, Chattopadhyaya *et al.*, *J. Org. Chem.*, 2009, 74, 118-134); and 4'-CH₂—C(=CH₂)-2' (and analogs thereof; see, *e.g.*, U.S. Patent No. 8,278,426). The entire contents of each of the foregoing are hereby incorporated herein by reference.

Additional representative U.S. Patents and U.S. Patent Publications that teach the preparation of locked nucleic acid nucleotides include, but are not limited to, the following: U.S. Patent Nos. 6,268,490; 6,525,191; 6,670,461; 6,770,748; 6,794,499; 6,998,484; 7,053,207; 7,034,133; 7,084,125; 7,399,845; 7,427,672; 7,569,686; 7,741,457; 8,022,193; 8,030,467; 8,278,425; 8,278,426; 8,278,283; 5 US 2008/0039618; and US 2009/0012281, the entire contents of each of which are hereby incorporated herein by reference.

Any of the foregoing bicyclic nucleosides can be prepared having one or more stereochemical sugar configurations including for example α -L-ribofuranose and β -D-ribofuranose (see WO 99/14226).

10 The RNA of an iRNA can also be modified to include one or more constrained ethyl nucleotides. As used herein, a "constrained ethyl nucleotide" or "cEt" is a locked nucleic acid comprising a bicyclic sugar moiety comprising a 4'-CH(CH₃)-O-2' bridge. In one embodiment, a constrained ethyl nucleotide is in the S conformation referred to herein as "S-cEt."

15 An iRNA of the invention may also include one or more "conformationally restricted nucleotides" ("CRN"). CRN are nucleotide analogs with a linker connecting the C2' and C4' carbons of ribose or the C3 and -C5' carbons of ribose. CRN lock the ribose ring into a stable conformation and increase the hybridization affinity to mRNA. The linker is of sufficient length to place the oxygen in an optimal position for stability and affinity resulting in less ribose ring puckering.

20 Representative publications that teach the preparation of certain of the above noted CRN include, but are not limited to, U.S. Patent Publication No. 2013/0190383; and PCT publication WO 2013/036868, the entire contents of each of which are hereby incorporated herein by reference.

25 In some embodiments, an iRNA of the invention comprises one or more monomers that are UNA (unlocked nucleic acid) nucleotides. UNA is unlocked acyclic nucleic acid, wherein any of the bonds of the sugar has been removed, forming an unlocked "sugar" residue. In one example, UNA also encompasses monomer with bonds between C1'-C4' have been removed (*i.e.* the covalent carbon-oxygen-carbon bond between the C1' and C4' carbons). In another example, the C2'-C3' bond (*i.e.* the covalent carbon-carbon bond between the C2' and C3' carbons) of the sugar has been removed (see *Nuc. Acids Symp. Series*, 52, 133-134 (2008) and Fluiter *et al.*, *Mol. Biosyst.*, 2009, 10, 1039 hereby incorporated by reference).

30 Representative U.S. publications that teach the preparation of UNA include, but are not limited to, U.S. Patent No. 8,314,227; and U.S. Patent Publication Nos. 2013/0096289; 2013/0011922; and 2011/0313020, the entire contents of each of which are hereby incorporated herein by reference.

Potentially stabilizing modifications to the ends of RNA molecules can include N- 35 (acetylaminocaproyl)-4-hydroxyprolinol (Hyp-C6-NHAc), N-(caproyl-4-hydroxyprolinol (Hyp-C6), N-(acetyl-4-hydroxyprolinol (Hyp-NHAc), thymidine-2'-O-deoxythymidine (ether), N-(aminocaproyl)-4-hydroxyprolinol (Hyp-C6-amino), 2-docosanoyl-uridine-3"-phosphate, inverted base dT(idT) and others. Disclosure of this modification can be found in PCT Publication No. WO 2011/005861.

Other modifications of the nucleotides of an iRNA of the invention include a 5' phosphate or 5' phosphate mimic, *e.g.*, a 5'-terminal phosphate or phosphate mimic on the antisense strand of an iRNA. Suitable phosphate mimics are disclosed in, for example U.S. Patent Publication No. 2012/0157511, the entire contents of which are incorporated herein by reference.

5

A. Modified iRNAs Comprising Motifs of the Invention

In certain aspects of the invention, the double stranded RNA agents of the invention include agents with chemical modifications as disclosed, for example, in WO2013/075035, the entire contents of each of which are incorporated herein by reference. WO2013/075035 provides motifs of three
10 identical modifications on three consecutive nucleotides into a sense strand or antisense strand of a dsRNAi agent, particularly at or near the cleavage site. In some embodiments, the sense strand and antisense strand of the dsRNAi agent may otherwise be completely modified. The introduction of these motifs interrupts the modification pattern, if present, of the sense or antisense strand. The dsRNAi agent may be optionally conjugated with a GalNAc derivative ligand, for instance on the
15 sense strand.

More specifically, when the sense strand and antisense strand of the double stranded RNA agent are completely modified to have one or more motifs of three identical modifications on three consecutive nucleotides at or near the cleavage site of at least one strand of a dsRNAi agent, the gene silencing activity of the dsRNAi agent was observed.

Accordingly, the invention provides double stranded RNA agents capable of inhibiting the
20 expression of a target gene (*i.e.*, APOC3 gene) *in vivo*. The RNAi agent comprises a sense strand and an antisense strand. Each strand of the RNAi agent may be, for example, 17-30 nucleotides in length, 25-30 nucleotides in length, 27-30 nucleotides in length, 19-25 nucleotides in length, 19-23 nucleotides in length, 19-21 nucleotides in length, 21-25 nucleotides in length, or 21-23 nucleotides in
25 length.

The sense strand and antisense strand typically form a duplex double stranded RNA ("dsRNA"), also referred to herein as "dsRNAi agent." The duplex region of a dsRNAi agent may be, for example, the duplex region can be 27-30 nucleotide pairs in length, 19-25 nucleotide pairs in length, 19-23 nucleotide pairs in length, 19- 21 nucleotide pairs in length, 21-25 nucleotide pairs in
30 length, or 21-23 nucleotide pairs in length. In another example, the duplex region is selected from 19, 20, 21, 22, 23, 24, 25, 26, and 27 nucleotides in length.

In certain embodiments, the dsRNAi agent may contain one or more overhang regions or capping groups at the 3'-end, 5'-end, or both ends of one or both strands. The overhang can be, independently, 1-6 nucleotides in length, for instance 2-6 nucleotides in length, 1-5 nucleotides in
35 length, 2-5 nucleotides in length, 1-4 nucleotides in length, 2-4 nucleotides in length, 1-3 nucleotides in length, 2-3 nucleotides in length, or 1-2 nucleotides in length. In certain embodiments, the overhang regions can include extended overhang regions as provided above. The overhangs can be the result of one strand being longer than the other, or the result of two strands of the same length being staggered. The overhang can form a mismatch with the target mRNA or it can be

complementary to the gene sequences being targeted or can be another sequence. The first and second strands can also be joined, *e.g.*, by additional bases to form a hairpin, or by other non-base linkers.

In certain embodiments, the nucleotides in the overhang region of the dsRNAi agent can each independently be a modified or unmodified nucleotide including, but not limited to 2'-sugar modified, such as, 2'-F, 2'-O-methyl, thymidine (T), 2'-O-methoxyethyl-5-methyluridine (Teo), 2'-O-methoxyethyladenosine (Aeo), 2'-O-methoxyethyl-5-methylcytidine (m5Ceo), and any combinations thereof.

For example, TT can be an overhang sequence for either end on either strand. The overhang can form a mismatch with the target mRNA or it can be complementary to the gene sequences being targeted or can be another sequence.

The 5'- or 3'- overhangs at the sense strand, antisense strand, or both strands of the dsRNAi agent may be phosphorylated. In some embodiments, the overhang region(s) contains two nucleotides having a phosphorothioate between the two nucleotides, where the two nucleotides can be the same or different. In some embodiments, the overhang is present at the 3'-end of the sense strand, antisense strand, or both strands. In some embodiments, this 3'-overhang is present in the antisense strand. In some embodiments, this 3'-overhang is present in the sense strand.

The dsRNAi agent may contain only a single overhang, which can strengthen the interference activity of the RNAi, without affecting its overall stability. For example, the single-stranded overhang may be located at the 3'- end of the sense strand or, alternatively, at the 3'-end of the antisense strand. The RNAi may also have a blunt end, located at the 5'-end of the antisense strand (or the 3'-end of the sense strand) or *vice versa*. Generally, the antisense strand of the dsRNAi agent has a nucleotide overhang at the 3'-end, and the 5'-end is blunt. While not wishing to be bound by theory, the asymmetric blunt end at the 5'-end of the antisense strand and 3'-end overhang of the antisense strand favor the guide strand loading into RISC process.

In certain embodiments, the dsRNAi agent is a double ended bluntmer of 19 nucleotides in length, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 7, 8, 9 from the 5' end. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5' end.

In other embodiments, the dsRNAi agent is a double ended bluntmer of 20 nucleotides in length, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 8, 9, 10 from the 5' end. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5' end.

In yet other embodiments, the dsRNAi agent is a double ended bluntmer of 21 nucleotides in length, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 9, 10, 11 from the 5' end. The antisense strand contains at least

one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5' end.

In certain embodiments, the dsRNAi agent comprises a 21 nucleotide sense strand and a 23 nucleotide antisense strand, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 9, 10, 11 from the 5' end; the antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5' end, wherein one end of the RNAi agent is blunt, while the other end comprises a 2 nucleotide overhang. Preferably, the 2 nucleotide overhang is at the 3'-end of the antisense strand.

When the 2 nucleotide overhang is at the 3'-end of the antisense strand, there may be two phosphorothioate internucleotide linkages between the terminal three nucleotides, wherein two of the three nucleotides are the overhang nucleotides, and the third nucleotide is a paired nucleotide next to the overhang nucleotide. In one embodiment, the RNAi agent additionally has two phosphorothioate internucleotide linkages between the terminal three nucleotides at both the 5'-end of the sense strand and at the 5'-end of the antisense strand. In certain embodiments, every nucleotide in the sense strand and the antisense strand of the dsRNAi agent, including the nucleotides that are part of the motifs are modified nucleotides. In certain embodiments each residue is independently modified with a 2'-O-methyl or 3'-fluoro, *e.g.*, in an alternating motif. Optionally, the dsRNAi agent further comprises a ligand (preferably GalNAc₃).

In certain embodiments, the dsRNAi agent comprises a sense and an antisense strand, wherein the sense strand is 25-30 nucleotide residues in length, wherein starting from the 5' terminal nucleotide (position 1) positions 1 to 23 of the first strand comprise at least 8 ribonucleotides; the antisense strand is 36-66 nucleotide residues in length and, starting from the 3' terminal nucleotide, comprises at least 8 ribonucleotides in the positions paired with positions 1-23 of sense strand to form a duplex; wherein at least the 3' terminal nucleotide of antisense strand is unpaired with sense strand, and up to 6 consecutive 3' terminal nucleotides are unpaired with sense strand, thereby forming a 3' single stranded overhang of 1-6 nucleotides; wherein the 5' terminus of antisense strand comprises from 10-30 consecutive nucleotides which are unpaired with sense strand, thereby forming a 10-30 nucleotide single stranded 5' overhang; wherein at least the sense strand 5' terminal and 3' terminal nucleotides are base paired with nucleotides of antisense strand when sense and antisense strands are aligned for maximum complementarity, thereby forming a substantially duplexed region between sense and antisense strands; and antisense strand is sufficiently complementary to a target RNA along at least 19 ribonucleotides of antisense strand length to reduce target gene expression when the double stranded nucleic acid is introduced into a mammalian cell; and wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides, where at least one of the motifs occurs at or near the cleavage site. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at or near the cleavage site.

In certain embodiments, the dsRNAi agent comprises sense and antisense strands, wherein the dsRNAi agent comprises a first strand having a length which is at least 25 and at most 29 nucleotides

and a second strand having a length which is at most 30 nucleotides with at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at position 11, 12, 13 from the 5' end; wherein the 3' end of the first strand and the 5' end of the second strand form a blunt end and the second strand is 1-4 nucleotides longer at its 3' end than the first strand, wherein the duplex region which is at least 25 nucleotides in length, and the second strand is sufficiently complementary to a target mRNA along at least 19 nucleotide of the second strand length to reduce target gene expression when the RNAi agent is introduced into a mammalian cell, and wherein Dicer cleavage of the dsRNAi agent preferentially results in an siRNA comprising the 3'-end of the second strand, thereby reducing expression of the target gene in the mammal. Optionally, the dsRNAi agent further comprises a ligand.

In certain embodiments, the sense strand of the dsRNAi agent contains at least one motif of three identical modifications on three consecutive nucleotides, where one of the motifs occurs at the cleavage site in the sense strand.

In certain embodiments, the antisense strand of the dsRNAi agent can also contain at least one motif of three identical modifications on three consecutive nucleotides, where one of the motifs occurs at or near the cleavage site in the antisense strand.

For a dsRNAi agent having a duplex region of 19-23 nucleotides in length, the cleavage site of the antisense strand is typically around the 10, 11, and 12 positions from the 5'-end. Thus the motifs of three identical modifications may occur at the 9, 10, 11 positions; the 10, 11, 12 positions; the 11, 12, 13 positions; the 12, 13, 14 positions; or the 13, 14, 15 positions of the antisense strand, the count starting from the first nucleotide from the 5'-end of the antisense strand, or, the count starting from the first paired nucleotide within the duplex region from the 5' - end of the antisense strand. The cleavage site in the antisense strand may also change according to the length of the duplex region of the dsRNAi agent from the 5'-end.

The sense strand of the dsRNAi agent may contain at least one motif of three identical modifications on three consecutive nucleotides at the cleavage site of the strand; and the antisense strand may have at least one motif of three identical modifications on three consecutive nucleotides at or near the cleavage site of the strand. When the sense strand and the antisense strand form a dsRNA duplex, the sense strand and the antisense strand can be so aligned that one motif of the three nucleotides on the sense strand and one motif of the three nucleotides on the antisense strand have at least one nucleotide overlap, *i.e.*, at least one of the three nucleotides of the motif in the sense strand forms a base pair with at least one of the three nucleotides of the motif in the antisense strand. Alternatively, at least two nucleotides may overlap, or all three nucleotides may overlap.

In some embodiments, the sense strand of the dsRNAi agent may contain more than one motif of three identical modifications on three consecutive nucleotides. The first motif may occur at or near the cleavage site of the strand and the other motifs may be a wing modification. The term "wing modification" herein refers to a motif occurring at another portion of the strand that is separated from the motif at or near the cleavage site of the same strand. The wing modification is either adjacent to the first motif or is separated by at least one or more nucleotides. When the motifs are immediately

adjacent to each other then the chemistries of the motifs are distinct from each other, and when the motifs are separated by one or more nucleotide then the chemistries can be the same or different. Two or more wing modifications may be present. For instance, when two wing modifications are present, each wing modification may occur at one end relative to the first motif which is at or near cleavage site or on either side of the lead motif.

Like the sense strand, the antisense strand of the dsRNAi agent may contain more than one motifs of three identical modifications on three consecutive nucleotides, with at least one of the motifs occurring at or near the cleavage site of the strand. This antisense strand may also contain one or more wing modifications in an alignment similar to the wing modifications that may be present on the sense strand.

In some embodiments, the wing modification on the sense strand or antisense strand of the dsRNAi agent typically does not include the first one or two terminal nucleotides at the 3'-end, 5'-end, or both ends of the strand.

In other embodiments, the wing modification on the sense strand or antisense strand of the dsRNAi agent typically does not include the first one or two paired nucleotides within the duplex region at the 3'-end, 5'-end, or both ends of the strand.

When the sense strand and the antisense strand of the dsRNAi agent each contain at least one wing modification, the wing modifications may fall on the same end of the duplex region, and have an overlap of one, two, or three nucleotides.

When the sense strand and the antisense strand of the dsRNAi agent each contain at least two wing modifications, the sense strand and the antisense strand can be so aligned that two modifications each from one strand fall on one end of the duplex region, having an overlap of one, two, or three nucleotides; two modifications each from one strand fall on the other end of the duplex region, having an overlap of one, two or three nucleotides; two modifications one strand fall on each side of the lead motif, having an overlap of one, two or three nucleotides in the duplex region.

In some embodiments, every nucleotide in the sense strand and antisense strand of the dsRNAi agent, including the nucleotides that are part of the motifs, may be modified. Each nucleotide may be modified with the same or different modification which can include one or more alteration of one or both of the non-linking phosphate oxygens or of one or more of the linking phosphate oxygens; alteration of a constituent of the ribose sugar, *e.g.*, of the 2'-hydroxyl on the ribose sugar; wholesale replacement of the phosphate moiety with "dephospho" linkers; modification or replacement of a naturally occurring base; and replacement or modification of the ribose-phosphate backbone.

As nucleic acids are polymers of subunits, many of the modifications occur at a position which is repeated within a nucleic acid, *e.g.*, a modification of a base, or a phosphate moiety, or a non-linking O of a phosphate moiety. In some cases the modification will occur at all of the subject positions in the nucleic acid but in many cases it will not. By way of example, a modification may only occur at a 3'- or 5' terminal position, may only occur in a terminal region, *e.g.*, at a position on a terminal nucleotide or in the last 2, 3, 4, 5, or 10 nucleotides of a strand. A modification may occur in

a double strand region, a single strand region, or in both. A modification may occur only in the double strand region of an RNA or may only occur in a single strand region of a RNA. For example, a phosphorothioate modification at a non-linking O position may only occur at one or both termini, may only occur in a terminal region, *e.g.*, at a position on a terminal nucleotide or in the last 2, 3, 4, 5, or 10 nucleotides of a strand, or may occur in double strand and single strand regions, particularly at termini. The 5'-end or ends can be phosphorylated.

It may be possible, *e.g.*, to enhance stability, to include particular bases in overhangs, or to include modified nucleotides or nucleotide surrogates, in single strand overhangs, *e.g.*, in a 5'- or 3'-overhang, or in both. For example, it can be desirable to include purine nucleotides in overhangs. In some embodiments all or some of the bases in a 3'- or 5'-overhang may be modified, *e.g.*, with a modification described herein. Modifications can include, *e.g.*, the use of modifications at the 2' position of the ribose sugar with modifications that are known in the art, *e.g.*, the use of deoxyribonucleotides, 2'-deoxy-2'-fluoro (2'-F) or 2'-O-methyl modified instead of the ribosugar of the nucleobase, and modifications in the phosphate group, *e.g.*, phosphorothioate modifications. Overhangs need not be homologous with the target sequence.

In some embodiments, each residue of the sense strand and antisense strand is independently modified with LNA, CRN, cET, UNA, HNA, CeNA, 2'-methoxyethyl, 2'-O-methyl, 2'-O-allyl, 2'-C-allyl, 2'-deoxy, 2'-hydroxyl, or 2'-fluoro. The strands can contain more than one modification. In one embodiment, each residue of the sense strand and antisense strand is independently modified with 2'-O-methyl or 2'-fluoro.

At least two different modifications are typically present on the sense strand and antisense strand. Those two modifications may be the 2'-O-methyl or 2'-fluoro modifications, or others.

In certain embodiments, the N_a or N_b comprise modifications of an alternating pattern. The term "alternating motif" as used herein refers to a motif having one or more modifications, each modification occurring on alternating nucleotides of one strand. The alternating nucleotide may refer to one per every other nucleotide or one per every three nucleotides, or a similar pattern. For example, if A, B and C each represent one type of modification to the nucleotide, the alternating motif can be "ABABABABABAB...", "AABBAABBAABB...", "AABAABAABAAB...", "AAABAAABAAAB...", "AAABBBAAABBB...", or "ABCABCABCABC...", *etc.*

The type of modifications contained in the alternating motif may be the same or different. For example, if A, B, C, D each represent one type of modification on the nucleotide, the alternating pattern, *i.e.*, modifications on every other nucleotide, may be the same, but each of the sense strand or antisense strand can be selected from several possibilities of modifications within the alternating motif such as "ABABAB...", "ACACAC...", "BDBDBD..." or "CDCDCD...", *etc.*

In some embodiments, the dsRNAi agent of the invention comprises the modification pattern for the alternating motif on the sense strand relative to the modification pattern for the alternating motif on the antisense strand is shifted. The shift may be such that the modified group of nucleotides of the sense strand corresponds to a differently modified group of nucleotides of the antisense strand and *vice versa*. For example, the sense strand when paired with the antisense strand in the dsRNA

duplex, the alternating motif in the sense strand may start with “ABABAB” from 5’ to 3’ of the strand and the alternating motif in the antisense strand may start with “BABABA” from 5’ to 3’ of the strand within the duplex region. As another example, the alternating motif in the sense strand may start with “AABBAABB” from 5’ to 3’ of the strand and the alternating motif in the antisense strand may start with “BBAABBAA” from 5’ to 3’ of the strand within the duplex region, so that there is a complete or partial shift of the modification patterns between the sense strand and the antisense strand.

In some embodiments, the dsRNAi agent comprises the pattern of the alternating motif of 2’-O-methyl modification and 2’-F modification on the sense strand initially has a shift relative to the pattern of the alternating motif of 2’-O-methyl modification and 2’-F modification on the antisense strand initially, *i.e.*, the 2’-O-methyl modified nucleotide on the sense strand base pairs with a 2’-F modified nucleotide on the antisense strand and vice versa. The 1 position of the sense strand may start with the 2’-F modification, and the 1 position of the antisense strand may start with the 2’-O-methyl modification.

The introduction of one or more motifs of three identical modifications on three consecutive nucleotides to the sense strand or antisense strand interrupts the initial modification pattern present in the sense strand or antisense strand. This interruption of the modification pattern of the sense or antisense strand by introducing one or more motifs of three identical modifications on three consecutive nucleotides to the sense or antisense strand may enhance the gene silencing activity against the target gene.

In some embodiments, when the motif of three identical modifications on three consecutive nucleotides is introduced to any of the strands, the modification of the nucleotide next to the motif is a different modification than the modification of the motif. For example, the portion of the sequence containing the motif is “...N_aYYYN_b...,” where “Y” represents the modification of the motif of three identical modifications on three consecutive nucleotide, and “N_a” and “N_b” represent a modification to the nucleotide next to the motif “YYY” that is different than the modification of Y, and where N_a and N_b can be the same or different modifications. Alternatively, N_a or N_b may be present or absent when there is a wing modification present.

The iRNA may further comprise at least one phosphorothioate or methylphosphonate internucleotide linkage. The phosphorothioate or methylphosphonate internucleotide linkage modification may occur on any nucleotide of the sense strand, antisense strand, or both strands in any position of the strand. For instance, the internucleotide linkage modification may occur on every nucleotide on the sense strand or antisense strand; each internucleotide linkage modification may occur in an alternating pattern on the sense strand or antisense strand; or the sense strand or antisense strand may contain both internucleotide linkage modifications in an alternating pattern. The alternating pattern of the internucleotide linkage modification on the sense strand may be the same or different from the antisense strand, and the alternating pattern of the internucleotide linkage modification on the sense strand may have a shift relative to the alternating pattern of the internucleotide linkage modification on the antisense strand. In one embodiment, a double-stranded RNAi agent comprises 6-8 phosphorothioate internucleotide linkages. In some embodiments, the

antisense strand comprises two phosphorothioate internucleotide linkages at the 5'-end and two phosphorothioate internucleotide linkages at the 3'-end, and the sense strand comprises at least two phosphorothioate internucleotide linkages at either the 5'-end or the 3'-end.

In some embodiments, the dsRNAi agent comprises a phosphorothioate or methylphosphonate internucleotide linkage modification in the overhang region. For example, the overhang region may contain two nucleotides having a phosphorothioate or methylphosphonate internucleotide linkage between the two nucleotides. Internucleotide linkage modifications also may be made to link the overhang nucleotides with the terminal paired nucleotides within the duplex region. For example, at least 2, 3, 4, or all the overhang nucleotides may be linked through phosphorothioate or methylphosphonate internucleotide linkage, and optionally, there may be additional phosphorothioate or methylphosphonate internucleotide linkages linking the overhang nucleotide with a paired nucleotide that is next to the overhang nucleotide. For instance, there may be at least two phosphorothioate internucleotide linkages between the terminal three nucleotides, in which two of the three nucleotides are overhang nucleotides, and the third is a paired nucleotide next to the overhang nucleotide. These terminal three nucleotides may be at the 3'-end of the antisense strand, the 3'-end of the sense strand, the 5'-end of the antisense strand, or the 5'-end of the antisense strand.

In some embodiments, the 2-nucleotide overhang is at the 3'-end of the antisense strand, and there are two phosphorothioate internucleotide linkages between the terminal three nucleotides, wherein two of the three nucleotides are the overhang nucleotides, and the third nucleotide is a paired nucleotide next to the overhang nucleotide. Optionally, the dsRNAi agent may additionally have two phosphorothioate internucleotide linkages between the terminal three nucleotides at both the 5'-end of the sense strand and at the 5'-end of the antisense strand.

In one embodiment, the dsRNAi agent comprises mismatch(es) with the target, within the duplex, or combinations thereof. The mismatch may occur in the overhang region or the duplex region. The base pair may be ranked on the basis of their propensity to promote dissociation or melting (*e.g.*, on the free energy of association or dissociation of a particular pairing, the simplest approach is to examine the pairs on an individual pair basis, though next neighbor or similar analysis can also be used). In terms of promoting dissociation: A:U is preferred over G:C; G:U is preferred over G:C; and I:C is preferred over G:C (I=inosine). Mismatches, *e.g.*, non-canonical or other than canonical pairings (as described elsewhere herein) are preferred over canonical (A:T, A:U, G:C) pairings; and pairings which include a universal base are preferred over canonical pairings.

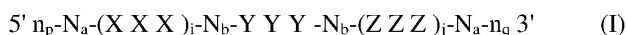
In certain embodiments, the dsRNAi agent comprises at least one of the first 1, 2, 3, 4, or 5 base pairs within the duplex regions from the 5'-end of the antisense strand independently selected from the group of: A:U, G:U, I:C, and mismatched pairs, *e.g.*, non-canonical or other than canonical pairings or pairings which include a universal base, to promote the dissociation of the antisense strand at the 5'-end of the duplex.

In certain embodiments, the nucleotide at the 1 position within the duplex region from the 5'-end in the antisense strand is selected from A, dA, dU, U, and dT. Alternatively, at least one of the

first 1, 2, or 3 base pair within the duplex region from the 5'-end of the antisense strand is an AU base pair. For example, the first base pair within the duplex region from the 5'-end of the antisense strand is an AU base pair.

In other embodiments, the nucleotide at the 3'-end of the sense strand is deoxy-thymine (dT) or the nucleotide at the 3'-end of the antisense strand is deoxy-thymine (dT). For example, there is a short sequence of deoxy-thymine nucleotides, for example, two dT nucleotides on the 3'-end of the sense, antisense strand, or both strands.

In certain embodiments, the sense strand sequence may be represented by formula (I):



wherein:

i and j are each independently 0 or 1;

p and q are each independently 0-6;

each N_a independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides;

each N_b independently represents an oligonucleotide sequence comprising 0-10 modified nucleotides;

each n_p and n_q independently represent an overhang nucleotide;

wherein N_b and Y do not have the same modification; and

XXX, YYY, and ZZZ each independently represent one motif of three identical modifications on three consecutive nucleotides. Preferably YYY is all 2'-F modified nucleotides.

In some embodiments, the N_a or N_b comprises modifications of alternating pattern.

In some embodiments, the YYY motif occurs at or near the cleavage site of the sense strand. For example, when the dsRNAi agent has a duplex region of 17-23 nucleotides in length, the YYY motif can occur at or the vicinity of the cleavage site (*e.g.*: can occur at positions 6, 7, 8; 7, 8, 9; 8, 9, 10; 9, 10, 11; 10, 11, 12; or 11, 12, 13) of the sense strand, the count starting from the first nucleotide, from the 5'-end; or optionally, the count starting at the first paired nucleotide within the duplex region, from the 5'-end.

In one embodiment, i is 1 and j is 0, or i is 0 and j is 1, or both i and j are 1. The sense strand can therefore be represented by the following formulas:

5' $n_p-N_a-YYY-N_b-ZZZ-N_a-n_q$ 3' (Ib);

5' $n_p-N_a-XXX-N_b-YYY-N_a-n_q$ 3' (Ic); or

5' $n_p-N_a-XXX-N_b-YYY-N_b-ZZZ-N_a-n_q$ 3' (Id).

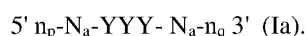
When the sense strand is represented by formula (Ib), N_b represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. Each N_a independently can represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the sense strand is represented as formula (Ic), N_b represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. Each N_a can independently represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the sense strand is represented as formula (Id), each N_b independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. Preferably, N_b is 0, 1, 2, 3, 4, 5, or 6. Each N_a can independently represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

5 Each of X, Y and Z may be the same or different from each other.

In other embodiments, i is 0 and j is 0, and the sense strand may be represented by the formula:



When the sense strand is represented by formula (Ia), each N_a independently can represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

10 In one embodiment, the antisense strand sequence of the RNAi may be represented by formula (II):



wherein:

15 k and l are each independently 0 or 1;

p' and q' are each independently 0-6;

each N_a' independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides;

each N_b' independently represents an oligonucleotide sequence comprising 0-10 modified nucleotides;

each n_p' and n_q' independently represent an overhang nucleotide;

wherein N_b' and Y' do not have the same modification; and

$X'X'X'$, $Y'Y'Y'$, and $Z'Z'Z'$ each independently represent one motif of three identical modifications on three consecutive nucleotides.

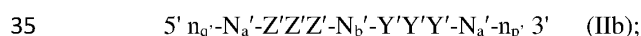
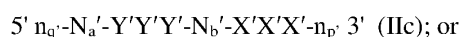
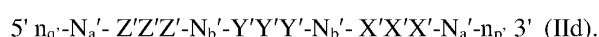
25 In some embodiments, the N_a' or N_b' comprises modifications of alternating pattern.

The $Y'Y'Y'$ motif occurs at or near the cleavage site of the antisense strand. For example, when the dsRNAi agent has a duplex region of 17-23 nucleotides in length, the $Y'Y'Y'$ motif can occur at positions 9, 10, 11; 10, 11, 12; 11, 12, 13; 12, 13, 14; or 13, 14, 15 of the antisense strand, with the count starting from the first nucleotide, from the 5'-end; or optionally, the count starting at the first paired nucleotide within the duplex region, from the 5'-end. Preferably, the $Y'Y'Y'$ motif occurs at positions 11, 12, 13.

In certain embodiments, $Y'Y'Y'$ motif is all 2'-OMe modified nucleotides.

In certain embodiments, k is 1 and l is 0, or k is 0 and l is 1, or both k and l are 1.

The antisense strand can therefore be represented by the following formulas:



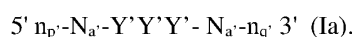
When the antisense strand is represented by formula (IIb), N_b' represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. Each N_a'

independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the antisense strand is represented as formula (IIc), N_b' represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. Each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the antisense strand is represented as formula (IId), each N_b' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. Each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides. Preferably, N_b is 0, 1, 2, 3, 4, 5, or 6.

In other embodiments, k is 0 and l is 0 and the antisense strand may be represented by the formula:



When the antisense strand is represented as formula (IIa), each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides. Each of X' , Y' and Z' may be the same or different from each other.

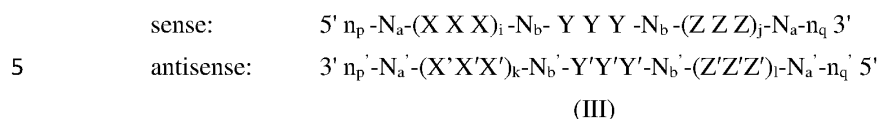
Each nucleotide of the sense strand and antisense strand may be independently modified with LNA, CRN, UNA, cEt, HNA, CeNA, 2'-methoxyethyl, 2'-O-methyl, 2'-O-allyl, 2'-C-allyl, 2'-hydroxyl, or 2'-fluoro. For example, each nucleotide of the sense strand and antisense strand is independently modified with 2'-O-methyl or 2'-fluoro. Each X , Y , Z , X' , Y' , and Z' , in particular, may represent a 2'-O-methyl modification or a 2'-fluoro modification.

In some embodiments, the sense strand of the dsRNAi agent may contain YYY motif occurring at 9, 10, and 11 positions of the strand when the duplex region is 21 nt, the count starting from the first nucleotide from the 5'-end, or optionally, the count starting at the first paired nucleotide within the duplex region, from the 5'-end; and Y represents 2'-F modification. The sense strand may additionally contain XXX motif or ZZZ motifs as wing modifications at the opposite end of the duplex region; and XXX and ZZZ each independently represents a 2'-OMe modification or 2'-F modification.

In some embodiments the antisense strand may contain $Y'Y'Y'$ motif occurring at positions 11, 12, 13 of the strand, the count starting from the first nucleotide from the 5'-end, or optionally, the count starting at the first paired nucleotide within the duplex region, from the 5'-end; and Y' represents 2'-O-methyl modification. The antisense strand may additionally contain $X'X'X'$ motif or $Z'Z'Z'$ motifs as wing modifications at the opposite end of the duplex region; and $X'X'X'$ and $Z'Z'Z'$ each independently represents a 2'-OMe modification or 2'-F modification.

The sense strand represented by any one of the above formulas (Ia), (Ib), (Ic), and (Id) forms a duplex with an antisense strand being represented by any one of formulas (IIa), (IIb), (IIc), and (IId), respectively.

Accordingly, the dsRNAi agents for use in the methods of the invention may comprise a sense strand and an antisense strand, each strand having 14 to 30 nucleotides, the iRNA duplex represented by formula (III):



wherein:

i, j, k, and l are each independently 0 or 1;

p, p', q, and q' are each independently 0-6;

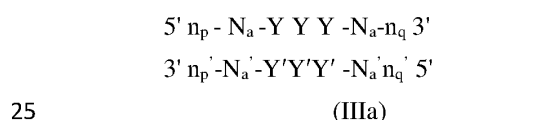
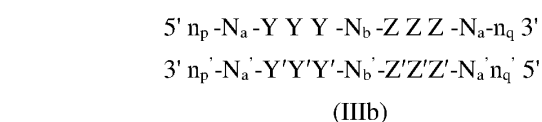
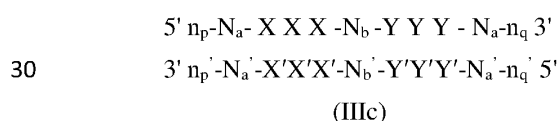
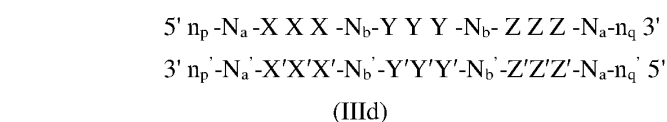
- 10 each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides;
each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 modified nucleotides;

- 15 wherein each n_p' , n_p , n_q' , and n_q , each of which may or may not be present, independently represents an overhang nucleotide; and

XXX, YYY, ZZZ, X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides.

- In one embodiment, i is 0 and j is 0; or i is 1 and j is 0; or i is 0 and j is 1; or both i and j are 0; or both i and j are 1. In another embodiment, k is 0 and l is 0; or k is 1 and l is 0; k is 0 and l is 1; or
20 both k and l are 0; or both k and l are 1.

Exemplary combinations of the sense strand and antisense strand forming an iRNA duplex include the formulas below:



- 35 When the dsRNAi agent is represented by formula (IIIa), each N_a independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the dsRNAi agent is represented by formula (IIIb), each N_b independently represents an oligonucleotide sequence comprising 1-10, 1-7, 1-5, or 1-4 modified nucleotides. Each N_a

independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the dsRNAi agent is represented as formula (IIIc), each N_b , N_b' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. Each N_a independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the dsRNAi agent is represented as formula (IIId), each N_b , N_b' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. Each N_a , N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides. Each of N_a , N_a' , N_b , and N_b' independently comprises modifications of alternating pattern.

Each of X, Y, and Z in formulas (III), (IIIa), (IIIb), (IIIc), and (IIId) may be the same or different from each other.

When the dsRNAi agent is represented by formula (III), (IIIa), (IIIb), (IIIc), and (IIId), at least one of the Y nucleotides may form a base pair with one of the Y' nucleotides. Alternatively, at least two of the Y nucleotides form base pairs with the corresponding Y' nucleotides; or all three of the Y nucleotides all form base pairs with the corresponding Y' nucleotides.

When the dsRNAi agent is represented by formula (IIIb) or (IIId), at least one of the Z nucleotides may form a base pair with one of the Z' nucleotides. Alternatively, at least two of the Z nucleotides form base pairs with the corresponding Z' nucleotides; or all three of the Z nucleotides all form base pairs with the corresponding Z' nucleotides.

When the dsRNAi agent is represented as formula (IIIc) or (IIId), at least one of the X nucleotides may form a base pair with one of the X' nucleotides. Alternatively, at least two of the X nucleotides form base pairs with the corresponding X' nucleotides; or all three of the X nucleotides all form base pairs with the corresponding X' nucleotides.

In certain embodiments, the modification on the Y nucleotide is different than the modification on the Y' nucleotide, the modification on the Z nucleotide is different than the modification on the Z' nucleotide, or the modification on the X nucleotide is different than the modification on the X' nucleotide.

In certain embodiments, when the dsRNAi agent is represented by formula (IIId), the N_a modifications are 2'-O-methyl or 2'-fluoro modifications. In other embodiments, when the RNAi agent is represented by formula (IIId), the N_a modifications are 2'-O-methyl or 2'-fluoro modifications and $n_p' > 0$ and at least one n_p' is linked to a neighboring nucleotide *via* phosphorothioate linkage. In yet other embodiments, when the RNAi agent is represented by formula (IIId), the N_a modifications are 2'-O-methyl or 2'-fluoro modifications, $n_p' > 0$ and at least one n_p' is linked to a neighboring nucleotide *via* phosphorothioate linkage, and the sense strand is conjugated to one or more GalNAc derivatives attached through a bivalent or trivalent branched linker (described below). In other embodiments, when the RNAi agent is represented by formula (IIId), the N_a modifications are 2'-O-methyl or 2'-fluoro modifications, $n_p' > 0$ and at least one n_p' is linked to a neighboring nucleotide *via*

phosphorothioate linkage, the sense strand comprises at least one phosphorothioate linkage, and the sense strand is conjugated to one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In some embodiments, when the dsRNAi agent is represented by formula (IIIa), the N_a modifications are 2'-O-methyl or 2'-fluoro modifications, $n_p' > 0$ and at least one n_p' is linked to a neighboring nucleotide *via* phosphorothioate linkage, the sense strand comprises at least one phosphorothioate linkage, and the sense strand is conjugated to one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In some embodiments, the dsRNAi agent is a multimer containing at least two duplexes represented by formula (III), (IIIa), (IIIb), (IIIc), and (IIId), wherein the duplexes are connected by a linker. The linker can be cleavable or non-cleavable. Optionally, the multimer further comprises a ligand. Each of the duplexes can target the same gene or two different genes; or each of the duplexes can target same gene at two different target sites.

In some embodiments, the dsRNAi agent is a multimer containing three, four, five, six, or more duplexes represented by formula (III), (IIIa), (IIIb), (IIIc), and (IIId), wherein the duplexes are connected by a linker. The linker can be cleavable or non-cleavable. Optionally, the multimer further comprises a ligand. Each of the duplexes can target the same gene or two different genes; or each of the duplexes can target same gene at two different target sites.

In one embodiment, two dsRNAi agents represented by at least one of formulas (III), (IIIa), (IIIb), (IIIc), and (IIId) are linked to each other at the 5' end, and one or both of the 3' ends, and are optionally conjugated to a ligand. Each of the agents can target the same gene or two different genes; or each of the agents can target same gene at two different target sites.

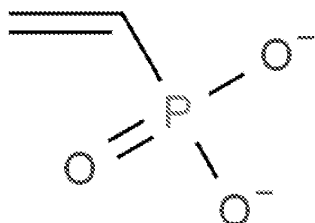
In certain embodiments, an RNAi agent of the invention may contain a low number of nucleotides containing a 2'-fluoro modification, *e.g.*, 10 or fewer nucleotides with 2'-fluoro modification. For example, the RNAi agent may contain 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 or 0 nucleotides with a 2'-fluoro modification. In a specific embodiment, the RNAi agent of the invention contains 10 nucleotides with a 2'-fluoro modification, *e.g.*, 4 nucleotides with a 2'-fluoro modification in the sense strand and 6 nucleotides with a 2'-fluoro modification in the antisense strand. In another specific embodiment, the RNAi agent of the invention contains 6 nucleotides with a 2'-fluoro modification, *e.g.*, 4 nucleotides with a 2'-fluoro modification in the sense strand and 2 nucleotides with a 2'-fluoro modification in the antisense strand.

In other embodiments, an RNAi agent of the invention may contain an ultra low number of nucleotides containing a 2'-fluoro modification, *e.g.*, 2 or fewer nucleotides containing a 2'-fluoro modification. For example, the RNAi agent may contain 2, 1 or 0 nucleotides with a 2'-fluoro modification. In a specific embodiment, the RNAi agent may contain 2 nucleotides with a 2'-fluoro modification, *e.g.*, 0 nucleotides with a 2'-fluoro modification in the sense strand and 2 nucleotides with a 2'-fluoro modification in the antisense strand.

Various publications describe multimeric iRNAs that can be used in the methods of the invention. Such publications include WO2007/091269, U.S. Patent No. 7,858,769, WO2010/141511,

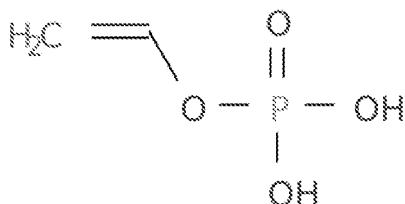
WO2007/117686, WO2009/014887, and WO2011/031520 the entire contents of each of which are hereby incorporated herein by reference.

In certain embodiments, the compositions and methods of the disclosure include a vinyl phosphonate (VP) modification of an RNAi agent as described herein. In exemplary embodiments, a vinyl phosphonate of the disclosure has the following structure:



A vinyl phosphonate of the instant disclosure may be attached to either the antisense or the sense strand of a dsRNA of the disclosure. In certain preferred embodiments, a vinyl phosphonate of the instant disclosure is attached to the antisense strand of a dsRNA, optionally at the 5' end of the antisense strand of the dsRNA.

Vinyl phosphate modifications are also contemplated for the compositions and methods of the instant disclosure. An exemplary vinyl phosphate structure is:



As described in more detail below, the iRNA that contains conjugations of one or more carbohydrate moieties to an iRNA can optimize one or more properties of the iRNA. In many cases, the carbohydrate moiety will be attached to a modified subunit of the iRNA. For example, the ribose sugar of one or more ribonucleotide subunits of a iRNA can be replaced with another moiety, *e.g.*, a non-carbohydrate (preferably cyclic) carrier to which is attached a carbohydrate ligand. A ribonucleotide subunit in which the ribose sugar of the subunit has been so replaced is referred to herein as a ribose replacement modification subunit (RRMS). A cyclic carrier may be a carbocyclic ring system, *i.e.*, all ring atoms are carbon atoms, or a heterocyclic ring system, *i.e.*, one or more ring atoms may be a heteroatom, *e.g.*, nitrogen, oxygen, sulfur. The cyclic carrier may be a monocyclic ring system, or may contain two or more rings, *e.g.* fused rings. The cyclic carrier may be a fully saturated ring system, or it may contain one or more double bonds.

The ligand may be attached to the polynucleotide *via* a carrier. The carriers include (i) at least one "backbone attachment point," preferably two "backbone attachment points" and (ii) at least one "tethering attachment point." A "backbone attachment point" as used herein refers to a functional

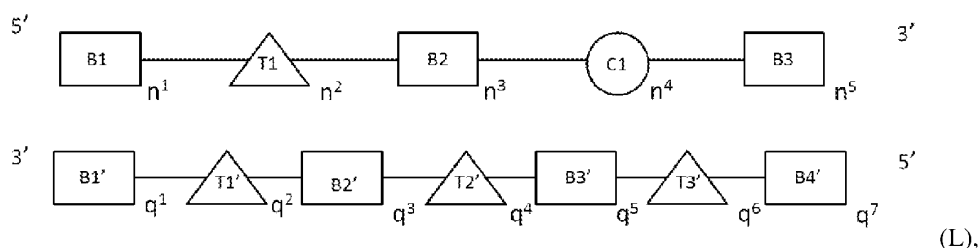
group, *e.g.* a hydroxyl group, or generally, a bond available for, and that is suitable for incorporation of the carrier into the backbone, *e.g.*, the phosphate, or modified phosphate, *e.g.*, sulfur containing, backbone, of a ribonucleic acid. A “tethering attachment point” (TAP) in some embodiments refers to a constituent ring atom of the cyclic carrier, *e.g.*, a carbon atom or a heteroatom (distinct from an atom which provides a backbone attachment point), that connects a selected moiety. The moiety can be, *e.g.*, a carbohydrate, *e.g.* monosaccharide, disaccharide, trisaccharide, tetrasaccharide, oligosaccharide, or polysaccharide. Optionally, the selected moiety is connected by an intervening tether to the cyclic carrier. Thus, the cyclic carrier will often include a functional group, *e.g.*, an amino group, or generally, provide a bond, that is suitable for incorporation or tethering of another chemical entity, *e.g.*, a ligand to the constituent ring.

The iRNA may be conjugated to a ligand *via* a carrier, wherein the carrier can be cyclic group or acyclic group; preferably, the cyclic group is selected from pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolane, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuryl, and decalin; preferably, the acyclic group is a serinol backbone or diethanolamine backbone.

i. Thermally Destabilizing Modifications

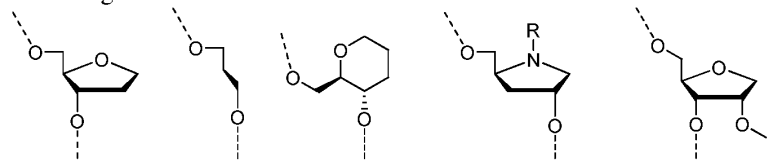
In certain embodiments, a dsRNA molecule can be optimized for RNA interference by incorporating thermally destabilizing modifications in the seed region of the antisense strand (*i.e.*, at positions 2-9 of the 5'-end of the antisense strand) to reduce or inhibit off-target gene silencing. It has been discovered that dsRNAs with an antisense strand comprising at least one thermally destabilizing modification of the duplex within the first 9 nucleotide positions, counting from the 5' end, of the antisense strand have reduced off-target gene silencing activity. Accordingly, in some embodiments, the antisense strand comprises at least one (*e.g.*, one, two, three, four, five or more) thermally destabilizing modification of the duplex within the first 9 nucleotide positions of the 5' region of the antisense strand. In some embodiments, one or more thermally destabilizing modification(s) of the duplex is/are located in positions 2-9, or preferably positions 4-8, from the 5'-end of the antisense strand. In some further embodiments, the thermally destabilizing modification(s) of the duplex is/are located at position 6, 7 or 8 from the 5'-end of the antisense strand. In still some further embodiments, the thermally destabilizing modification of the duplex is located at position 7 from the 5'-end of the antisense strand. The term “thermally destabilizing modification(s)” includes modification(s) that would result with a dsRNA with a lower overall melting temperature (T_m) (preferably a T_m with one, two, three or four degrees lower than the T_m of the dsRNA without having such modification(s)). In some embodiments, the thermally destabilizing modification of the duplex is located at position 2, 3, 4, 5 or 9 from the 5'-end of the antisense strand.

An iRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides. The RNAi agent may be represented by formula (L):

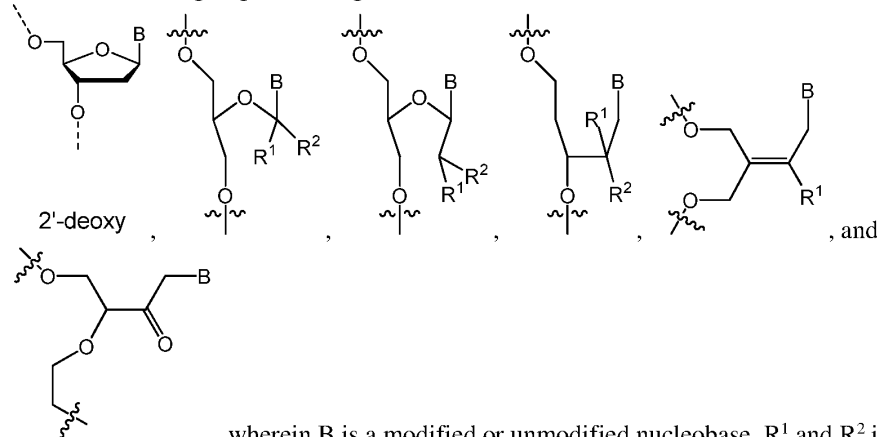


In formula (L), B1, B2, B3, B1', B2', B3', and B4' each are independently a nucleotide containing a modification selected from the group consisting of 2'-O-alkyl, 2'-substituted alkoxy, 2'-substituted alkyl, 2'-halo, ENA, and BNA/LNA. In one embodiment, B1, B2, B3, B1', B2', B3', and B4' each contain 2'-OMe modifications. In one embodiment, B1, B2, B3, B1', B2', B3', and B4' each contain 2'-OMe or 2'-F modifications. In one embodiment, at least one of B1, B2, B3, B1', B2', B3', and B4' contain 2'-O-N-methylacetamido (2'-O-NMA) modification.

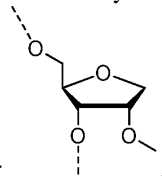
C1 is a thermally destabilizing nucleotide placed at a site opposite to the seed region of the antisense strand (*i.e.*, at positions 2-8 of the 5'-end of the antisense strand). For example, C1 is at a position of the sense strand that pairs with a nucleotide at positions 2-8 of the 5'-end of the antisense strand. In one example, C1 is at position 15 from the 5'-end of the sense strand. C1 nucleotide bears the thermally destabilizing modification which can include abasic modification; mismatch with the opposing nucleotide in the duplex; and sugar modification such as 2'-deoxy modification or acyclic nucleotide *e.g.*, unlocked nucleic acids (UNA) or glycerol nucleic acid (GNA). In one embodiment, C1 has thermally destabilizing modification selected from the group consisting of: i) mismatch with the opposing nucleotide in the antisense strand; ii) abasic modification selected from the group consisting of:



selected from the group consisting of:



H, halogen, OR₃, or alkyl; and R₃ is H, alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or sugar. In one embodiment, the thermally destabilizing modification in C1 is a mismatch selected from the group consisting of G:G, G:A, G:U, G:T, A:A, A:C, C:C, C:U, C:T, U:U, T:T, and U:T; and optionally, at least one nucleobase in the mismatch pair is a 2'-deoxy nucleobase. In one example, the thermally



- destabilizing modification in C1 is GNA or T1, T1', T2', and T3' each independently represent a nucleotide comprising a modification providing the nucleotide a steric bulk that is less or equal to the steric bulk of a 2'-OMe modification. A steric bulk refers to the sum of steric effects of a modification. Methods for determining steric effects of a modification of a nucleotide are known to one skilled in the art. The modification can be at the 2' position of a ribose sugar of the nucleotide, or a modification to a non-ribose nucleotide, acyclic nucleotide, or the backbone of the nucleotide that is similar or equivalent to the 2' position of the ribose sugar, and provides the nucleotide a steric bulk that is less than or equal to the steric bulk of a 2'-OMe modification. For example, T1, T1', T2', and T3' are each independently selected from DNA, RNA, LNA, 2'-F, and 2'-F-5'-methyl. In one embodiment, T1 is DNA. In one embodiment, T1' is DNA, RNA or LNA. In one embodiment, T2' is DNA or RNA. In one embodiment, T3' is DNA or RNA.

n^1 , n^3 , and q^1 are independently 4 to 15 nucleotides in length.

n^5 , q^3 , and q^7 are independently 1-6 nucleotide(s) in length.

n^4 , q^2 , and q^6 are independently 1-3 nucleotide(s) in length; alternatively, n^4 is 0.

- q^5 is independently 0-10 nucleotide(s) in length.

n^2 and q^4 are independently 0-3 nucleotide(s) in length.

Alternatively, n^4 is 0-3 nucleotide(s) in length.

- In one embodiment, n^4 can be 0. In one example, n^4 is 0, and q^2 and q^6 are 1. In another example, n^4 is 0, and q^2 and q^6 are 1, with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

In one embodiment, n^4 , q^2 , and q^6 are each 1.

- In one embodiment, n^2 , n^4 , q^2 , q^4 , and q^6 are each 1.

In one embodiment, C1 is at position 14-17 of the 5'-end of the sense strand, when the sense strand is 19-22 nucleotides in length, and n^4 is 1. In one embodiment, C1 is at position 15 of the 5'-end of the sense strand

- In one embodiment, T3' starts at position 2 from the 5' end of the antisense strand. In one example, T3' is at position 2 from the 5' end of the antisense strand and q^6 is equal to 1.

In one embodiment, T1' starts at position 14 from the 5' end of the antisense strand. In one example, T1' is at position 14 from the 5' end of the antisense strand and q^2 is equal to 1.

In an exemplary embodiment, T3' starts from position 2 from the 5' end of the antisense strand and T1' starts from position 14 from the 5' end of the antisense strand. In one example, T3' starts from position 2 from the 5' end of the antisense strand and q^6 is equal to 1 and T1' starts from position 14 from the 5' end of the antisense strand and q^2 is equal to 1.

In one embodiment, T1' and T3' are separated by 11 nucleotides in length (*i.e.* not counting the T1' and T3' nucleotides).

In one embodiment, T1' is at position 14 from the 5' end of the antisense strand. In one example, T1' is at position 14 from the 5' end of the antisense strand and q^2 is equal to 1, and the modification at the 2' position or positions in a non-ribose, acyclic or backbone that provide less steric bulk than a 2'-OMe ribose.

In one embodiment, T3' is at position 2 from the 5' end of the antisense strand. In one example, T3' is at position 2 from the 5' end of the antisense strand and q^6 is equal to 1, and the modification at the 2' position or positions in a non-ribose, acyclic or backbone that provide less than or equal to steric bulk than a 2'-OMe ribose.

In one embodiment, T1 is at the cleavage site of the sense strand. In one example, T1 is at position 11 from the 5' end of the sense strand, when the sense strand is 19-22 nucleotides in length, and n^2 is 1. In an exemplary embodiment, T1 is at the cleavage site of the sense strand at position 11 from the 5' end of the sense strand, when the sense strand is 19-22 nucleotides in length, and n^2 is 1,

In one embodiment, T2' starts at position 6 from the 5' end of the antisense strand. In one example, T2' is at positions 6-10 from the 5' end of the antisense strand, and q^4 is 1.

In an exemplary embodiment, T1 is at the cleavage site of the sense strand, for instance, at position 11 from the 5' end of the sense strand, when the sense strand is 19-22 nucleotides in length, and n^2 is 1; T1' is at position 14 from the 5' end of the antisense strand, and q^2 is equal to 1, and the modification to T1' is at the 2' position of a ribose sugar or at positions in a non-ribose, acyclic or backbone that provide less steric bulk than a 2'-OMe ribose; T2' is at positions 6-10 from the 5' end of the antisense strand, and q^4 is 1; and T3' is at position 2 from the 5' end of the antisense strand, and q^6 is equal to 1, and the modification to T3' is at the 2' position or at positions in a non-ribose, acyclic or backbone that provide less than or equal to steric bulk than a 2'-OMe ribose.

In one embodiment, T2' starts at position 8 from the 5' end of the antisense strand. In one example, T2' starts at position 8 from the 5' end of the antisense strand, and q^4 is 2.

In one embodiment, T2' starts at position 9 from the 5' end of the antisense strand. In one example, T2' is at position 9 from the 5' end of the antisense strand, and q^4 is 1.

In one embodiment, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 1, B3' is 2'-OMe or 2'-F, q^5 is 6, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage

modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

In one embodiment, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 1, B3' is 2'-OMe or 2'-F, q^5 is 6, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 6, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 7, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 6, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 7, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 1, B3' is 2'-OMe or 2'-F, q^5 is 6, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 1, B3' is 2'-OMe or 2'-F, q^5 is 6, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand

(counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 5, T2' is 2'-F, q^4 is 1, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; optionally with at least 2 additional TT at the 3'-end of the antisense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 5, T2' is 2'-F, q^4 is 1, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; optionally with at least 2 additional TT at the 3'-end of the antisense strand; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end).

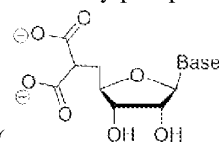
In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

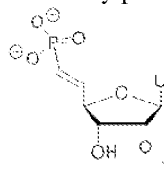
In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

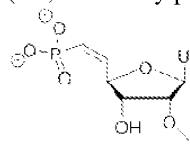
The RNAi agent can comprise a phosphorus-containing group at the 5'-end of the sense strand or antisense strand. The 5'-end phosphorus-containing group can be 5'-end phosphate (5'-P), 5'-end phosphorothioate (5'-PS), 5'-end phosphorodithioate (5'-PS₂), 5'-end vinylphosphonate (5'-VP), 5'-end methylphosphonate (MePhos), or 5'-deoxy-5'-C-malonyl (



VP), 5'-end methylphosphonate (MePhos), or 5'-deoxy-5'-C-malonyl (). When the 5'-end phosphorus-containing group is 5'-end vinylphosphonate (5'-VP), the 5'-VP can be either



5'-E-VP isomer (*i.e.*, *trans*-vinylphosphate,), 5'-Z-VP isomer (*i.e.*, *cis*-



vinylphosphate,), or mixtures thereof.

- 15 In one embodiment, the RNAi agent comprises a phosphorus-containing group at the 5'-end of the sense strand. In one embodiment, the RNAi agent comprises a phosphorus-containing group at the 5'-end of the antisense strand.

In one embodiment, the RNAi agent comprises a 5'-P. In one embodiment, the RNAi agent comprises a 5'-P in the antisense strand.

- 20 In one embodiment, the RNAi agent comprises a 5'-PS. In one embodiment, the RNAi agent comprises a 5'-PS in the antisense strand.

In one embodiment, the RNAi agent comprises a 5'-VP. In one embodiment, the RNAi agent comprises a 5'-VP in the antisense strand. In one embodiment, the RNAi agent comprises a 5'-E-VP in the antisense strand. In one embodiment, the RNAi agent comprises a 5'-Z-VP in the antisense strand.

- 25 In one embodiment, the RNAi agent comprises a 5'-PS₂. In one embodiment, the RNAi agent comprises a 5'-PS₂ in the antisense strand.

In one embodiment, the RNAi agent comprises a 5'-PS₂. In one embodiment, the RNAi agent comprises a 5'-deoxy-5'-C-malonyl in the antisense strand.

- 30 In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4,

T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1. The RNAi agent also comprises a 5'-PS.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1. The RNAi agent also comprises a 5'-P.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1. The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-E-VP, 5'-Z-VP, or combination thereof.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1. The RNAi agent also comprises a 5'-PS₂.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1. The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-P.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-PS.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense

strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-E-VP, 5'-Z-VP, or

5 combination thereof.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand
10 (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-PS₂.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage
20 modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The
25 RNAi agent also comprises a 5'-P.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The dsRNA agent also comprises a 5'-PS.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-E-VP, 5'-Z-VP, or combination thereof.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The RNAi agent also comprises a 5'-PS₂.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F,

q^3 is 4, q^4 is 0, $B3'$ is 2'-OMe or 2'-F, q^5 is 7, $T3'$ is 2'-F, q^6 is 1, $B4'$ is 2'-OMe, and q^7 is 1. The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, $B1'$ is 2'-OMe or 2'-F, q^1 is 9, $T1'$ is 2'-F, q^2 is 1, $B2'$ is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, $B3'$ is 2'-OMe or 2'-F, q^5 is 7, $T3'$ is 2'-F, q^6 is 1, $B4'$ is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-P.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, $B1'$ is 2'-OMe or 2'-F, q^1 is 9, $T1'$ is 2'-F, q^2 is 1, $B2'$ is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, $B3'$ is 2'-OMe or 2'-F, q^5 is 7, $T3'$ is 2'-F, q^6 is 1, $B4'$ is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-PS.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, $B1'$ is 2'-OMe or 2'-F, q^1 is 9, $T1'$ is 2'-F, q^2 is 1, $B2'$ is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, $B3'$ is 2'-OMe or 2'-F, q^5 is 7, $T3'$ is 2'-F, q^6 is 1, $B4'$ is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-E-VP, 5'-Z-VP, or combination thereof.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, $B1'$ is 2'-OMe or 2'-F, q^1 is 9, $T1'$ is 2'-F, q^2 is 1, $B2'$ is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, $B3'$ is 2'-OMe or 2'-F, q^5 is 7, $T3'$ is 2'-F, q^6 is 1, $B4'$ is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-PS₂.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, $B1'$ is 2'-OMe or 2'-F, q^1 is 9, $T1'$ is 2'-F, q^2 is 1, $B2'$ is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, $B3'$ is 2'-OMe or 2'-F, q^5 is 7, $T3'$ is 2'-F, q^6 is 1, $B4'$ is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The RNAi agent also comprises a 5'-P.

- 5 In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The RNAi agent also comprises a 5'-PS.

- 10 In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-E-VP, 5'-Z-VP, or combination thereof.

- 15 In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The dsRNAi RNA agent also comprises a 5'-PS₂.

- 20 In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl.

- 25 In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-P.

- 30 In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-PS.

- 35 In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F,

q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-E-VP, 5'-Z-VP, or combination thereof.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-PS₂.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The RNAi agent also comprises a 5'-P.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The RNAi agent also comprises a 5'-PS.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-E-VP, 5'-Z-VP, or combination thereof.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The RNAi agent also comprises a 5'-PS₂.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl.

- 5 In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage
10 modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-P.

- In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F,
15 q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The
20 RNAi agent also comprises a 5'-PS.

- In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand
25 (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-E-VP, 5'-Z-VP, or combination thereof.

- In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand
30 (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-PS₂.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two

phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-P and a targeting ligand. In one embodiment, the 5'-P is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-PS and a targeting ligand. In one embodiment, the 5'-PS is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-VP (*e.g.*, a 5'-E-VP, 5'-Z-VP, or combination thereof), and a targeting ligand.

In one embodiment, the 5'-VP is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide

linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-PS₂ and a targeting ligand. In one embodiment, the 5'-PS₂ is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

5 In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl and a targeting ligand. In one embodiment, the 5'-deoxy-5'-C-malonyl is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

15 In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-P and a targeting ligand. In one embodiment, the 5'-P is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

25 In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-PS and a targeting ligand. In one embodiment, the 5'-PS is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

30 In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-VP (*e.g.*, a

5'-E-VP, 5'-Z-VP, or combination thereof) and a targeting ligand. In one embodiment, the 5'-VP is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-PS₂ and a targeting ligand. In one embodiment, the 5'-PS₂ is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl and a targeting ligand. In one embodiment, the 5'-deoxy-5'-C-malonyl is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-P and a targeting ligand. In one embodiment, the 5'-P is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-PS and a targeting ligand. In one embodiment, the 5'-PS is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-VP (*e.g.*, a 5'-E-VP, 5'-Z-VP, or combination thereof) and a targeting ligand. In one embodiment, the 5'-VP is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-PS₂ and a targeting ligand. In one embodiment, the 5'-PS₂ is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl and a targeting ligand. In one embodiment, the 5'-deoxy-5'-C-malonyl is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-P and a targeting ligand. In one embodiment, the 5'-P is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-PS and a targeting ligand. In one embodiment, the 5'-PS is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-VP (*e.g.*, a 5'-E-VP, 5'-Z-VP, or combination thereof) and a targeting ligand. In one embodiment, the 5'-VP is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-PS₂ and a targeting ligand. In one embodiment, the 5'-PS₂ is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'-F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl and a targeting ligand. In one embodiment, the 5'-deoxy-5'-C-malonyl is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In a particular embodiment, an RNAi agent of the present invention comprises:

- (a) a sense strand having:
- (i) a length of 21 nucleotides;
 - (ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker; and
 - (iii) 2'-F modifications at positions 1, 3, 5, 7, 9 to 11, 13, 17, 19, and 21, and 2'-OMe modifications at positions 2, 4, 6, 8, 12, 14 to 16, 18, and 20 (counting from the 5' end);

and

- (b) an antisense strand having:
- (i) a length of 23 nucleotides;
 - (ii) 2'-OMe modifications at positions 1, 3, 5, 9, 11 to 13, 15, 17, 19, 21, and 23, and 2'-F modifications at positions 2, 4, 6 to 8, 10, 14, 16, 18, 20, and 22 (counting from the 5' end); and
 - (iii) phosphorothioate internucleotide linkages between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

wherein the dsRNA agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In another particular embodiment, an RNAi agent of the present invention comprises:

- (a) a sense strand having:
- (i) a length of 21 nucleotides;
 - (ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;
 - (iii) 2'-F modifications at positions 1, 3, 5, 7, 9 to 11, 13, 15, 17, 19, and 21, and 2'-OMe modifications at positions 2, 4, 6, 8, 12, 14, 16, 18, and 20 (counting from the 5' end);

and

- (iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

- (b) an antisense strand having:
- (i) a length of 23 nucleotides;
 - (ii) 2'-OMe modifications at positions 1, 3, 5, 7, 9, 11 to 13, 15, 17, 19, and 21 to 23, and 2'-F modifications at positions 2, 4, 6, 8, 10, 14, 16, 18, and 20 (counting from the 5' end); and
 - (iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

wherein the RNAi agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In another particular embodiment, a RNAi agent of the present invention comprises:

- (a) a sense strand having:
- (i) a length of 21 nucleotides;
 - (ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;
 - (iii) 2'-OMe modifications at positions 1 to 6, 8, 10, and 12 to 21, 2'-F modifications at positions 7, and 9, and a deoxy-nucleotide (*e.g.* dT) at position 11 (counting from the 5' end); and
 - (iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);
- and
- (b) an antisense strand having:
- (i) a length of 23 nucleotides;
 - (ii) 2'-OMe modifications at positions 1, 3, 7, 9, 11, 13, 15, 17, and 19 to 23, and 2'-F modifications at positions 2, 4 to 6, 8, 10, 12, 14, 16, and 18 (counting from the 5' end); and
 - (iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);
- wherein the RNAi agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In another particular embodiment, a RNAi agent of the present invention comprises:

- (a) a sense strand having:
- (i) a length of 21 nucleotides;
 - (ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;
 - (iii) 2'-OMe modifications at positions 1 to 6, 8, 10, 12, 14, and 16 to 21, and 2'-F modifications at positions 7, 9, 11, 13, and 15; and
 - (iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);
- and
- (b) an antisense strand having:
- (i) a length of 23 nucleotides;
 - (ii) 2'-OMe modifications at positions 1, 5, 7, 9, 11, 13, 15, 17, 19, and 21 to 23, and 2'-F modifications at positions 2 to 4, 6, 8, 10, 12, 14, 16, 18, and 20 (counting from the 5' end); and
 - (iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

wherein the RNAi agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In another particular embodiment, a RNAi agent of the present invention comprises:

- (a) a sense strand having:
 - 5 (i) a length of 21 nucleotides;
 - (ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;
 - (iii) 2'-OMe modifications at positions 1 to 9, and 12 to 21, and 2'-F modifications at positions 10, and 11; and
 - 10 (iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);
- and
- (b) an antisense strand having:
 - 15 (i) a length of 23 nucleotides;
 - (ii) 2'-OMe modifications at positions 1, 3, 5, 7, 9, 11 to 13, 15, 17, 19, and 21 to 23, and 2'-F modifications at positions 2, 4, 6, 8, 10, 14, 16, 18, and 20 (counting from the 5' end); and
 - (iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and
 - 20 between nucleotide positions 22 and 23 (counting from the 5' end);

wherein the RNAi agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In another particular embodiment, a RNAi agent of the present invention comprises:

- (a) a sense strand having:
 - 25 (i) a length of 21 nucleotides;
 - (ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;
 - (iii) 2'-F modifications at positions 1, 3, 5, 7, 9 to 11, and 13, and 2'-OMe modifications at positions 2, 4, 6, 8, 12, and 14 to 21; and
 - 30 (iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);
- and
- (b) an antisense strand having:
 - (i) a length of 23 nucleotides;
 - 35 (ii) 2'-OMe modifications at positions 1, 3, 5 to 7, 9, 11 to 13, 15, 17 to 19, and 21 to 23, and 2'-F modifications at positions 2, 4, 8, 10, 14, 16, and 20 (counting from the 5' end); and
 - (iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between

nucleotide positions 22 and 23 (counting from the 5' end);
wherein the RNAi agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In another particular embodiment, a RNAi agent of the present invention comprises:

- 5 (a) a sense strand having:
- (i) a length of 21 nucleotides;
 - (ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;
 - (iii) 2'-OMe modifications at positions 1, 2, 4, 6, 8, 12, 14, 15, 17, and 19 to 21, and 2'-F
 - 10 modifications at positions 3, 5, 7, 9 to 11, 13, 16, and 18; and
 - (iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);
- and
- (b) an antisense strand having:
- 15 (i) a length of 25 nucleotides;
 - (ii) 2'-OMe modifications at positions 1, 4, 6, 7, 9, 11 to 13, 15, 17, and 19 to 23, 2'-F modifications at positions 2, 3, 5, 8, 10, 14, 16, and 18, and desoxy-nucleotides (*e.g.* dT) at positions 24 and 25 (counting from the 5' end); and
 - (iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2,
 - 20 between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

wherein the RNAi agents have a four nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In another particular embodiment, a RNAi agent of the present invention comprises:

- 25 (a) a sense strand having:
- (i) a length of 21 nucleotides;
 - (ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;
 - (iii) 2'-OMe modifications at positions 1 to 6, 8, and 12 to 21, and 2'-F modifications at
 - 30 positions 7, and 9 to 11; and
 - (iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);
- and
- (b) an antisense strand having:
- 35 (i) a length of 23 nucleotides;
 - (ii) 2'-OMe modifications at positions 1, 3 to 5, 7, 8, 10 to 13, 15, and 17 to 23, and 2'-F modifications at positions 2, 6, 9, 14, and 16 (counting from the 5' end); and

(iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

wherein the RNAi agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In another particular embodiment, a RNAi agent of the present invention comprises:

(a) a sense strand having:

- (i) a length of 21 nucleotides;
- (ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;
- (iii) 2'-OMe modifications at positions 1 to 6, 8, and 12 to 21, and 2'-F modifications at positions 7, and 9 to 11; and
- (iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

(b) an antisense strand having:

- (i) a length of 23 nucleotides;
- (ii) 2'-OMe modifications at positions 1, 3 to 5, 7, 10 to 13, 15, and 17 to 23, and 2'-F modifications at positions 2, 6, 8, 9, 14, and 16 (counting from the 5' end); and
- (iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

wherein the RNAi agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In another particular embodiment, a RNAi agent of the present invention comprises:

(a) a sense strand having:

- (i) a length of 19 nucleotides;
- (ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;
- (iii) 2'-OMe modifications at positions 1 to 4, 6, and 10 to 19, and 2'-F modifications at positions 5, and 7 to 9; and
- (iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

(b) an antisense strand having:

- (i) a length of 21 nucleotides;
- (ii) 2'-OMe modifications at positions 1, 3 to 5, 7, 10 to 13, 15, and 17 to 21, and 2'-F modifications at positions 2, 6, 8, 9, 14, and 16 (counting from the 5' end); and

- (iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 19 and 20, and between nucleotide positions 20 and 21 (counting from the 5' end);

wherein the RNAi agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In certain embodiments, the iRNA for use in the methods of the invention is an agent selected from agents listed in any one of Tables 2-5, 14, and 15. These agents may further comprise a ligand.

III. iRNAs Conjugated to Ligands

Another modification of the RNA of an iRNA of the invention involves chemically linking to the iRNA one or more ligands, moieties or conjugates that enhance the activity, cellular distribution, or cellular uptake of the iRNA *e.g.*, into a cell. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger *et al.*, *Proc. Natl. Acad. Sci. USA*, 1989, 86: 6553-6556). In other embodiments, the ligand is cholic acid (Manoharan *et al.*, *Biorg. Med. Chem. Lett.*, 1994, 4:1053-1060), a thioether, *e.g.*, beryl-S-tritylthiol (Manoharan *et al.*, *Ann. N.Y. Acad. Sci.*, 1992, 660:306-309; Manoharan *et al.*, *Biorg. Med. Chem. Lett.*, 1993, 3:2765-2770), a thiocholesterol (Oberhauser *et al.*, *Nucl. Acids Res.*, 1992, 20:533-538), an aliphatic chain, *e.g.*, dodecandiol or undecyl residues (Saison-Behmoaras *et al.*, *EMBO J*, 1991, 10:1111-1118; Kabanov *et al.*, *FEBS Lett.*, 1990, 259:327-330; Svinarchuk *et al.*, *Biochimie*, 1993, 75:49-54), a phospholipid, *e.g.*, dihexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-phosphonate (Manoharan *et al.*, *Tetrahedron Lett.*, 1995, 36:3651-3654; Shea *et al.*, *Nucl. Acids Res.*, 1990, 18:3777-3783), a polyamine or a polyethylene glycol chain (Manoharan *et al.*, *Nucleosides & Nucleotides*, 1995, 14:969-973), or adamantane acetic acid (Manoharan *et al.*, *Tetrahedron Lett.*, 1995, 36:3651-3654), a palmityl moiety (Mishra *et al.*, *Biochim. Biophys. Acta*, 1995, 1264:229-237), or an octadecylamine or hexylamino-carbonyloxycholesterol moiety (Crooke *et al.*, *J. Pharmacol. Exp. Ther.*, 1996, 277:923-937).

In certain embodiments, a ligand alters the distribution, targeting, or lifetime of an iRNA agent into which it is incorporated. In preferred embodiments a ligand provides an enhanced affinity for a selected target, *e.g.*, molecule, cell or cell type, compartment, *e.g.*, a cellular or organ compartment, tissue, organ or region of the body, as, *e.g.*, compared to a species absent such a ligand. Preferred ligands do not take part in duplex pairing in a duplexed nucleic acid.

Ligands can include a naturally occurring substance, such as a protein (*e.g.*, human serum albumin (HSA), low-density lipoprotein (LDL), or globulin); carbohydrate (*e.g.*, a dextran, pullulan, chitin, chitosan, inulin, cyclodextrin, N-acetylglucosamine, N-acetylgalactosamine, or hyaluronic acid); or a lipid. The ligand can also be a recombinant or synthetic molecule, such as a synthetic polymer, *e.g.*, a synthetic polyamino acid. Examples of polyamino acids include polyamino acid is a polylysine (PLL), poly L-aspartic acid, poly L-glutamic acid, styrene-maleic acid anhydride copolymer, poly(L-lactide-co-glycolide) copolymer, divinyl ether-maleic anhydride copolymer, N-(2-hydroxypropyl)methacrylamide copolymer (HMPA), polyethylene glycol (PEG), polyvinyl alcohol

(PVA), polyurethane, poly(2-ethylacrylic acid), N-isopropylacrylamide polymers, or polyphosphazine. Example of polyamines include: polyethylenimine, polylysine (PLL), spermine, spermidine, polyamine, pseudopeptide-polyamine, peptidomimetic polyamine, dendrimer polyamine, arginine, amidine, protamine, cationic lipid, cationic porphyrin, quaternary salt of a polyamine, or an alpha helical peptide.

Ligands can also include targeting groups, *e.g.*, a cell or tissue targeting agent, *e.g.*, a lectin, glycoprotein, lipid or protein, *e.g.*, an antibody, that binds to a specified cell type such as a kidney cell. A targeting group can be a thyrotropin, melanotropin, lectin, glycoprotein, surfactant protein A, Mucin carbohydrate, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-glucosamine multivalent mannose, multivalent fucose, glycosylated polyaminoacids, multivalent galactose, transferrin, bisphosphonate, polyglutamate, polyaspartate, a lipid, cholesterol, a steroid, bile acid, folate, vitamin B12, vitamin A, biotin, or an RGD peptide or RGD peptide mimetic. In certain embodiments, the ligand is a multivalent galactose, *e.g.*, an N-acetyl-galactosamine.

Other examples of ligands include dyes, intercalating agents (*e.g.* acridines), cross-linkers (*e.g.* psoralene, mitomycin C), porphyrins (TPPC4, texaphyrin, Sapphyrin), polycyclic aromatic hydrocarbons (*e.g.*, phenazine, dihydrophenazine), artificial endonucleases (*e.g.* EDTA), lipophilic molecules, *e.g.*, cholesterol, cholic acid, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-Bis-O(hexadecyl)glycerol, geranyloxyhexyl group, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid, O3-(oleoyl)lithocholic acid, O3-(oleoyl)cholenic acid, dimethoxytrityl, or phenoxazine) and peptide conjugates (*e.g.*, antennapedia peptide, Tat peptide), alkylating agents, phosphate, amino, mercapto, PEG (*e.g.*, PEG-40K), MPEG, [MPEG]₂, polyamino, alkyl, substituted alkyl, radiolabeled markers, enzymes, haptens (*e.g.* biotin), transport/absorption facilitators (*e.g.*, aspirin, vitamin E, folic acid), synthetic ribonucleases (*e.g.*, imidazole, bisimidazole, histamine, imidazole clusters, acridine-imidazole conjugates, Eu³⁺ complexes of tetraazamacrocycles), dinitrophenyl, HRP, or AP.

Ligands can be proteins, *e.g.*, glycoproteins, or peptides, *e.g.*, molecules having a specific affinity for a co-ligand, or antibodies *e.g.*, an antibody, that binds to a specified cell type such as a hepatic cell. Ligands can also include hormones and hormone receptors. They can also include non-peptidic species, such as lipids, lectins, carbohydrates, vitamins, cofactors, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-glucosamine multivalent mannose, or multivalent fucose. The ligand can be, for example, a lipopolysaccharide, an activator of p38 MAP kinase, or an activator of NF-κB.

The ligand can be a substance, *e.g.*, a drug, which can increase the uptake of the iRNA agent into the cell, for example, by disrupting the cell's cytoskeleton, *e.g.*, by disrupting the cell's microtubules, microfilaments, or intermediate filaments. The drug can be, for example, taxol, vincristine, vinblastine, cytochalasin, nocodazole, japlakinolide, latrunculin A, phalloidin, swinholid A, indanocine, or myoservin.

In some embodiments, a ligand attached to an iRNA as described herein acts as a pharmacokinetic modulator (PK modulator). PK modulators include lipophiles, bile acids, steroids,

phospholipid analogues, peptides, protein binding agents, PEG, vitamins, *etc.* Exemplary PK modulators include, but are not limited to, cholesterol, fatty acids, cholic acid, lithocholic acid, dialkylglycerides, diacylglyceride, phospholipids, sphingolipids, naproxen, ibuprofen, vitamin E, biotin. Oligonucleotides that comprise a number of phosphorothioate linkages are also known to bind to serum protein, thus short oligonucleotides, *e.g.*, oligonucleotides of about 5 bases, 10 bases, 15 bases, or 20 bases, comprising multiple of phosphorothioate linkages in the backbone are also amenable to the present invention as ligands (*e.g.* as PK modulating ligands). In addition, aptamers that bind serum components (*e.g.* serum proteins) are also suitable for use as PK modulating ligands in the embodiments described herein.

Ligand-conjugated iRNAs of the invention may be synthesized by the use of an oligonucleotide that bears a pendant reactive functionality, such as that derived from the attachment of a linking molecule onto the oligonucleotide (described below). This reactive oligonucleotide may be reacted directly with commercially-available ligands, ligands that are synthesized bearing any of a variety of protecting groups, or ligands that have a linking moiety attached thereto.

The oligonucleotides used in the conjugates of the present invention may be conveniently and routinely made through the well-known technique of solid-phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems® (Foster City, Calif.). Any other methods for such synthesis known in the art may additionally or alternatively be employed. It is also known to use similar techniques to prepare other oligonucleotides, such as the phosphorothioates and alkylated derivatives.

In the ligand-conjugated iRNAs and ligand-molecule bearing sequence-specific linked nucleosides of the present invention, the oligonucleotides and oligonucleosides may be assembled on a suitable DNA synthesizer utilizing standard nucleotide or nucleoside precursors, or nucleotide or nucleoside conjugate precursors that already bear the linking moiety, ligand-nucleotide or nucleoside-conjugate precursors that already bear the ligand molecule, or non-nucleoside ligand-bearing building blocks.

When using nucleotide-conjugate precursors that already bear a linking moiety, the synthesis of the sequence-specific linked nucleosides is typically completed, and the ligand molecule is then reacted with the linking moiety to form the ligand-conjugated oligonucleotide. In some embodiments, the oligonucleotides or linked nucleosides of the present invention are synthesized by an automated synthesizer using phosphoramidites derived from ligand-nucleoside conjugates in addition to the standard phosphoramidites and non-standard phosphoramidites that are commercially available and routinely used in oligonucleotide synthesis.

A. Lipid Conjugates

In certain embodiments, the ligand or conjugate is a lipid or lipid-based molecule. Such a lipid or lipid-based molecule preferably binds a serum protein, *e.g.*, human serum albumin (HSA). An HSA binding ligand allows for distribution of the conjugate to a target tissue, *e.g.*, a non-kidney target tissue of the body. For example, the target tissue can be the liver, including parenchymal cells

of the liver. Other molecules that can bind HSA can also be used as ligands. For example, naproxen or aspirin can be used. A lipid or lipid-based ligand can (a) increase resistance to degradation of the conjugate, (b) increase targeting or transport into a target cell or cell membrane, or (c) can be used to adjust binding to a serum protein, *e.g.*, HSA.

5 A lipid based ligand can be used to inhibit, *e.g.*, control the binding of the conjugate to a target tissue. For example, a lipid or lipid-based ligand that binds to HSA more strongly will be less likely to be targeted to the kidney and therefore less likely to be cleared from the body. A lipid or lipid-based ligand that binds to HSA less strongly can be used to target the conjugate to the kidney.

In certain embodiments, the lipid based ligand binds HSA. Preferably, it binds HSA with a
10 sufficient affinity such that the conjugate will be preferably distributed to a non-kidney tissue. However, it is preferred that the affinity not be so strong that the HSA-ligand binding cannot be reversed.

In other embodiments, the lipid based ligand binds HSA weakly or not at all, such that the conjugate will be preferably distributed to the kidney. Other moieties that target to kidney cells can
15 also be used in place of, or in addition to, the lipid based ligand.

In another aspect, the ligand is a moiety, *e.g.*, a vitamin, which is taken up by a target cell, *e.g.*, a proliferating cell. These are particularly useful for treating disorders characterized by unwanted cell proliferation, *e.g.*, of the malignant or non-malignant type, *e.g.*, cancer cells. Exemplary vitamins include vitamin A, E, and K. Other exemplary vitamins include are B vitamin,
20 *e.g.*, folic acid, B12, riboflavin, biotin, pyridoxal or other vitamins or nutrients taken up by target cells such as liver cells. Also included are HSA and low density lipoprotein (LDL).

B. Cell Permeation Agents

In another aspect, the ligand is a cell-permeation agent, preferably a helical cell-permeation
25 agent. Preferably, the agent is amphipathic. An exemplary agent is a peptide such as tat or antennopodia. If the agent is a peptide, it can be modified, including a peptidylmimetic, invertomers, non-peptide or pseudo-peptide linkages, and use of D-amino acids. The helical agent is preferably an alpha-helical agent, which preferably has a lipophilic and a lipophobic phase.

The ligand can be a peptide or peptidomimetic. A peptidomimetic (also referred to herein as
30 an oligopeptidomimetic) is a molecule capable of folding into a defined three-dimensional structure similar to a natural peptide. The attachment of peptide and peptidomimetics to iRNA agents can affect pharmacokinetic distribution of the iRNA, such as by enhancing cellular recognition and absorption. The peptide or peptidomimetic moiety can be about 5-50 amino acids long, *e.g.*, about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 amino acids long.

35 A peptide or peptidomimetic can be, for example, a cell permeation peptide, cationic peptide, amphipathic peptide, or hydrophobic peptide (*e.g.*, consisting primarily of Tyr, Trp, or Phe). The peptide moiety can be a dendrimer peptide, constrained peptide or crosslinked peptide. In another alternative, the peptide moiety can include a hydrophobic membrane translocation sequence (MTS). An exemplary hydrophobic MTS-containing peptide is RFGF having the amino acid sequence

- AAVALLPAVLLALLAP (SEQ ID NO: 18). An RFGF analogue (*e.g.*, amino acid sequence AALLPVLLAAP (SEQ ID NO:19) containing a hydrophobic MTS can also be a targeting moiety. The peptide moiety can be a “delivery” peptide, which can carry large polar molecules including peptides, oligonucleotides, and protein across cell membranes. For example, sequences from the HIV
- 5 Tat protein (GRKKRRQRRRPPQ (SEQ ID NO:20) and the *Drosophila* Antennapedia protein (RQIKIWFQNRRMKWKK (SEQ ID NO:21) have been found to be capable of functioning as delivery peptides. A peptide or peptidomimetic can be encoded by a random sequence of DNA, such as a peptide identified from a phage-display library, or one-bead-one-compound (OBOC) combinatorial library (Lam *et al.*, *Nature*, 354:82-84, 1991). Examples of a peptide or
- 10 peptidomimetic tethered to a dsRNA agent *via* an incorporated monomer unit for cell targeting purposes is an arginine-glycine-aspartic acid (RGD)-peptide, or RGD mimic. A peptide moiety can range in length from about 5 amino acids to about 40 amino acids. The peptide moieties can have a structural modification, such as to increase stability or direct conformational properties. Any of the structural modifications described below can be utilized.
- 15 An RGD peptide for use in the compositions and methods of the invention may be linear or cyclic, and may be modified, *e.g.*, glycosylated or methylated, to facilitate targeting to a specific tissue(s). RGD-containing peptides and peptidomimetics may include D-amino acids, as well as synthetic RGD mimics. In addition to RGD, one can use other moieties that target the integrin ligand. Preferred conjugates of this ligand target PECAM-1 or VEGF.
- 20 A “cell permeation peptide” is capable of permeating a cell, *e.g.*, a microbial cell, such as a bacterial or fungal cell, or a mammalian cell, such as a human cell. A microbial cell-permeating peptide can be, for example, an α -helical linear peptide (*e.g.*, LL-37 or Ceropin P1), a disulfide bond-containing peptide (*e.g.*, α -defensin, β -defensin or bactenecin), or a peptide containing only one or two dominating amino acids (*e.g.*, PR-39 or indolicidin). A cell permeation peptide can also include a
- 25 nuclear localization signal (NLS). For example, a cell permeation peptide can be a bipartite amphipathic peptide, such as MPG, which is derived from the fusion peptide domain of HIV-1 gp41 and the NLS of SV40 large T antigen (Simeoni *et al.*, *Nucl. Acids Res.* 31:2717-2724, 2003).

C. Carbohydrate Conjugates

- 30 In some embodiments of the compositions and methods of the invention, an iRNA further comprises a carbohydrate. The carbohydrate conjugated iRNA is advantageous for the *in vivo* delivery of nucleic acids, as well as compositions suitable for *in vivo* therapeutic use, as described herein. As used herein, “carbohydrate” refers to a compound which is either a carbohydrate *per se* made up of one or more monosaccharide units having at least 6 carbon atoms (which can be linear,
- 35 branched or cyclic) with an oxygen, nitrogen or sulfur atom bonded to each carbon atom; or a compound having as a part thereof a carbohydrate moiety made up of one or more monosaccharide units each having at least six carbon atoms (which can be linear, branched or cyclic), with an oxygen, nitrogen or sulfur atom bonded to each carbon atom. Representative carbohydrates include the sugars (mono-, di-, tri-, and oligosaccharides containing from about 4, 5, 6, 7, 8, or 9 monosaccharide units),

and polysaccharides such as starches, glycogen, cellulose and polysaccharide gums. Specific monosaccharides include C5 and above (*e.g.*, C5, C6, C7, or C8) sugars; di- and trisaccharides include sugars having two or three monosaccharide units (*e.g.*, C5, C6, C7, or C8).

5 In certain embodiments, a carbohydrate conjugate for use in the compositions and methods of the invention is a monosaccharide.

In certain embodiments, the monosaccharide is an N-acetylgalactosamine (GalNAc). GalNAc conjugates, which comprise one or more N-acetylgalactosamine (GalNAc) derivatives, are described, for example, in US 8,106,022, the entire content of which is hereby incorporated herein by reference. In some embodiments, the GalNAc conjugate serves as a ligand that targets the iRNA to particular
10 cells. In some embodiments, the GalNAc conjugate targets the iRNA to liver cells, *e.g.*, by serving as a ligand for the asialoglycoprotein receptor of liver cells (*e.g.*, hepatocytes).

In some embodiments, the carbohydrate conjugate comprises one or more GalNAc derivatives. The GalNAc derivatives may be attached *via* a linker, *e.g.*, a bivalent or trivalent branched linker. In some embodiments the GalNAc conjugate is conjugated to the 3' end of the sense
15 strand. In some embodiments, the GalNAc conjugate is conjugated to the iRNA agent (*e.g.*, to the 3' end of the sense strand) *via* a linker, *e.g.*, a linker as described herein. In some embodiments the GalNAc conjugate is conjugated to the 5' end of the sense strand. In some embodiments, the GalNAc conjugate is conjugated to the iRNA agent (*e.g.*, to the 5' end of the sense strand) *via* a linker, *e.g.*, a linker as described herein.

20 In certain embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a monovalent linker. In some embodiments, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a bivalent linker. In yet other embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a trivalent linker. In other embodiments of the invention, the GalNAc or GalNAc
25 derivative is attached to an iRNA agent of the invention *via* a tetravalent linker.

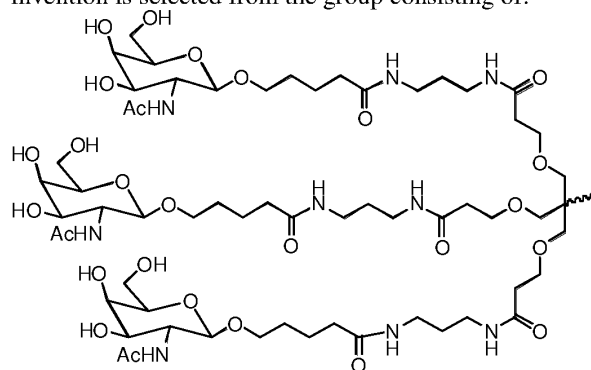
In certain embodiments, the double stranded RNAi agents of the invention comprise one GalNAc or GalNAc derivative attached to the iRNA agent. In certain embodiments, the double stranded RNAi agents of the invention comprise a plurality (*e.g.*, 2, 3, 4, 5, or 6) GalNAc or GalNAc derivatives, each independently attached to a plurality of nucleotides of the double stranded RNAi
30 agent through a plurality of monovalent linkers.

In some embodiments, for example, when the two strands of an iRNA agent of the invention are part of one larger molecule connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming a hairpin loop comprising, a plurality of unpaired nucleotides, each unpaired nucleotide within the hairpin loop may independently
35 comprise a GalNAc or GalNAc derivative attached *via* a monovalent linker. The hairpin loop may also be formed by an extended overhang in one strand of the duplex.

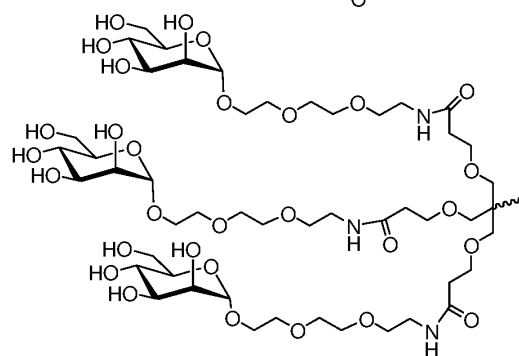
In some embodiments, for example, when the two strands of an iRNA agent of the invention are part of one larger molecule connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming a hairpin loop comprising, a

plurality of unpaired nucleotides, each unpaired nucleotide within the hairpin loop may independently comprise a GalNAc or GalNAc derivative attached *via* a monovalent linker. The hairpin loop may also be formed by an extended overhang in one strand of the duplex.

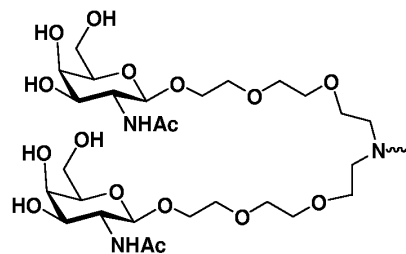
In one embodiment, a carbohydrate conjugate for use in the compositions and methods of the
5 invention is selected from the group consisting of:



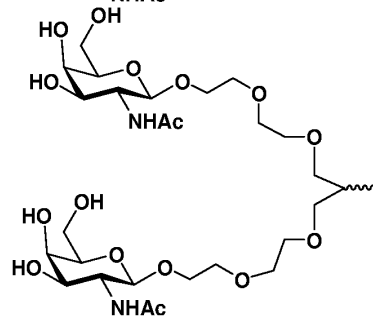
Formula II,



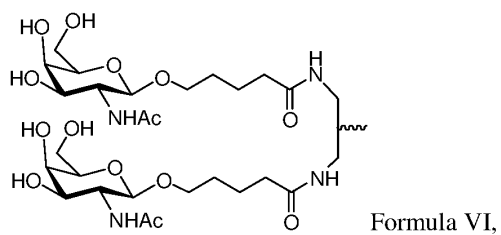
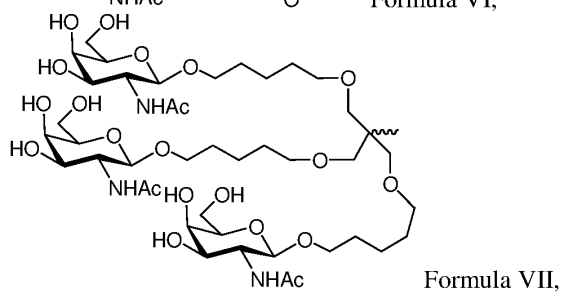
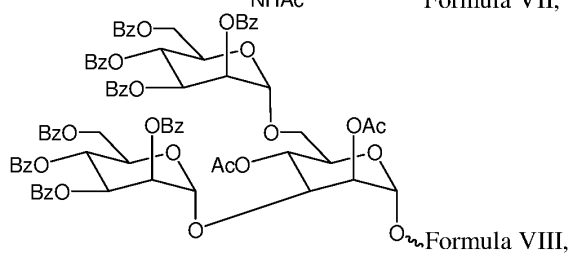
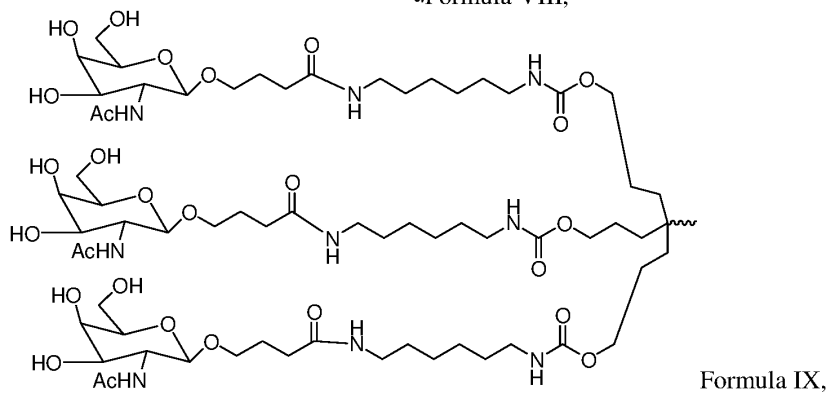
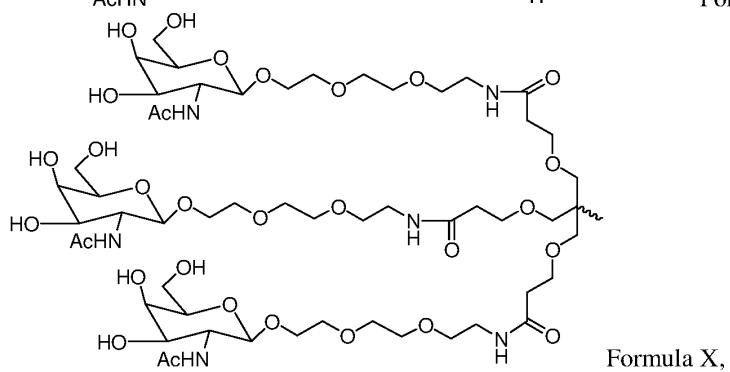
Formula III,

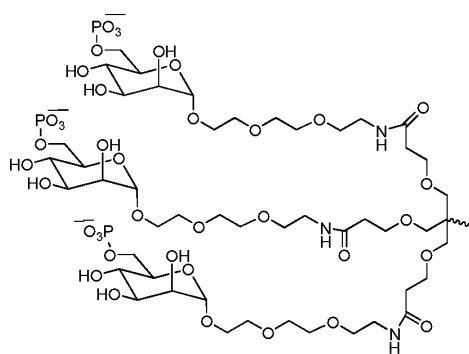


Formula IV,

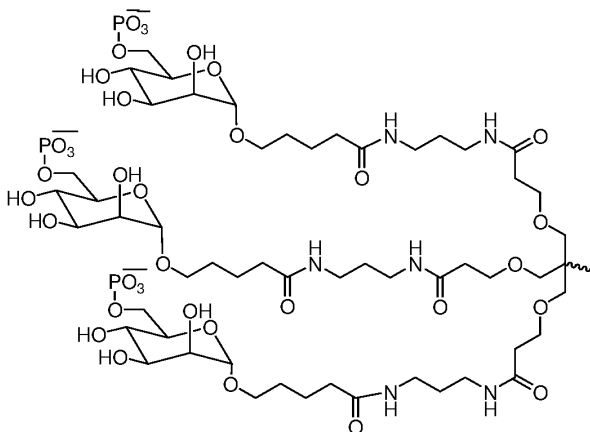


Formula V,

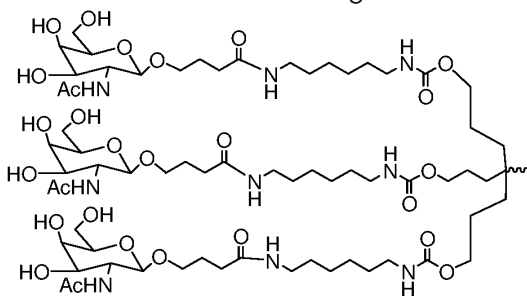




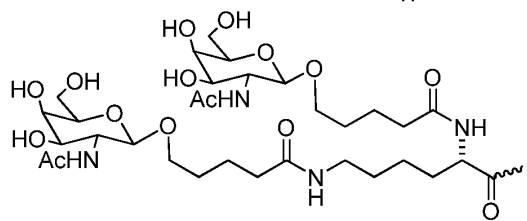
Formula XI,



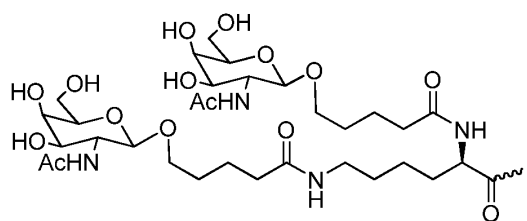
Formula XII,



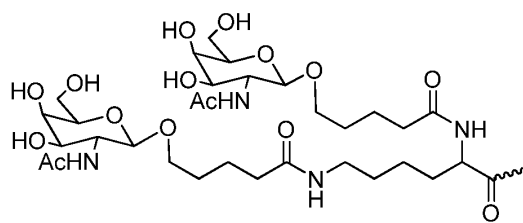
Formula XIII,



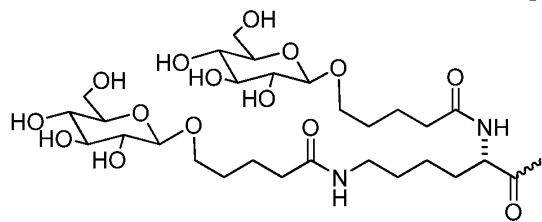
Formula XIV,



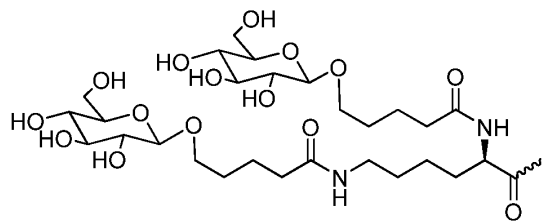
Formula XV,



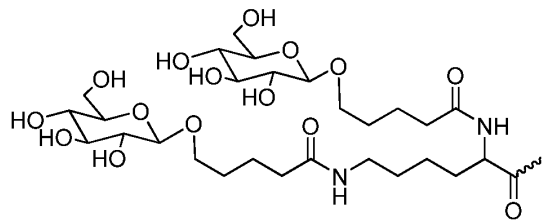
Formula XVI,



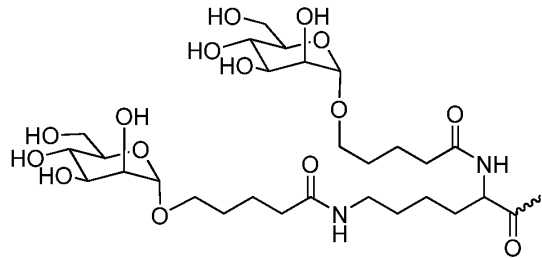
Formula XVII,



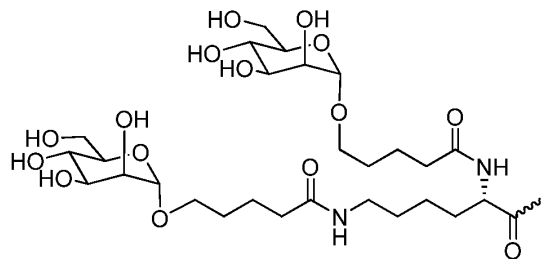
Formula XVIII,



Formula XIX,

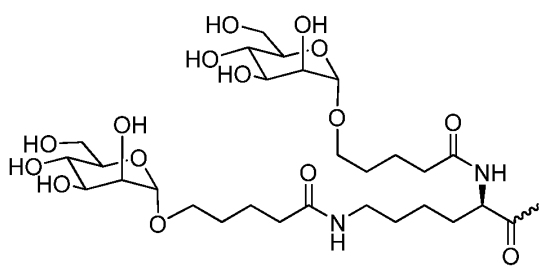


Formula XX,

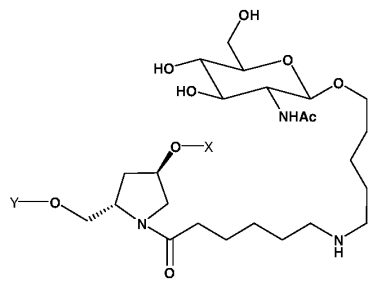


Formula XXI,

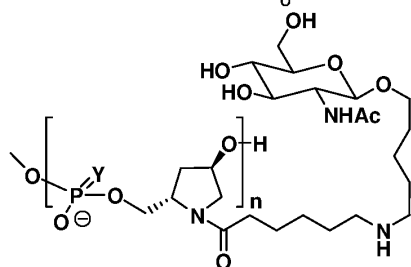
5



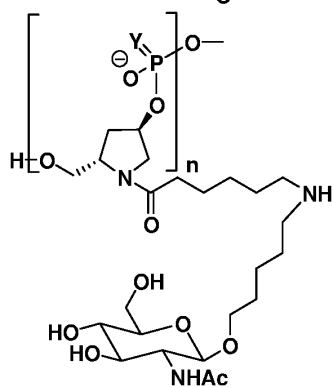
Formula XXII,



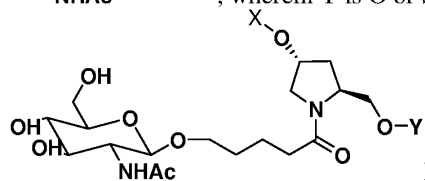
Formula XXIII;



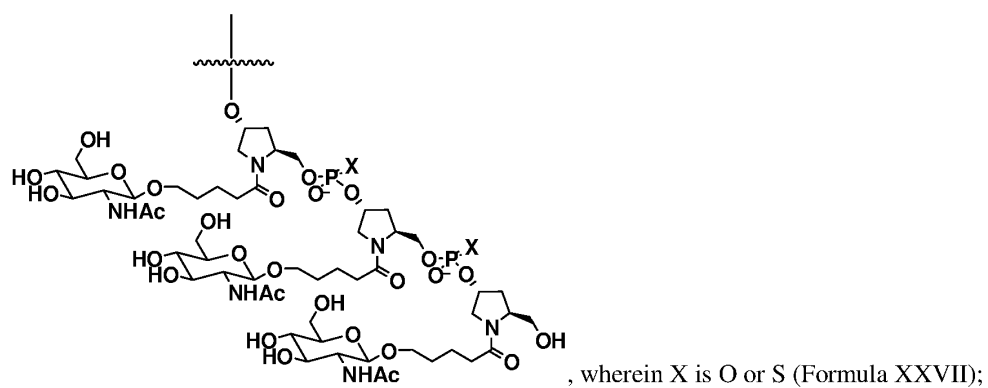
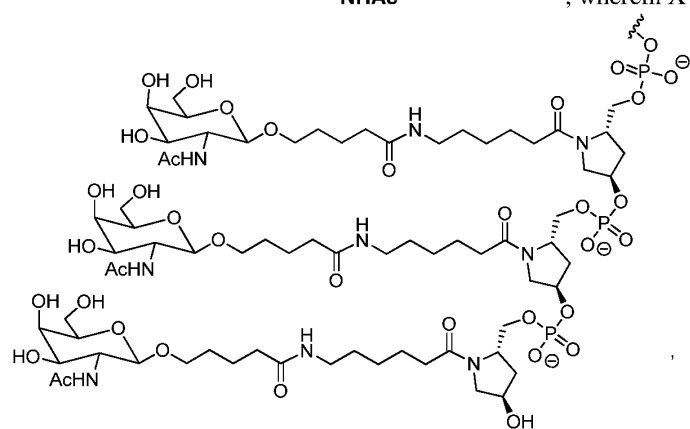
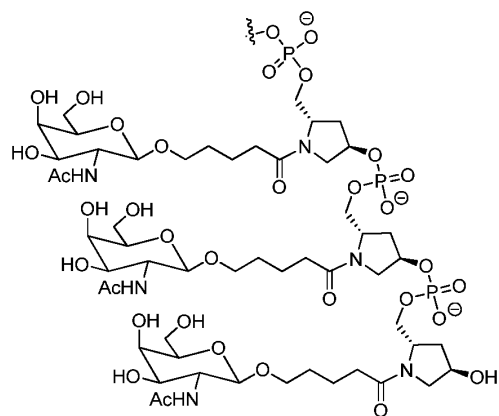
, wherein Y is O or S and n is 3 -6 (Formula XXIV);



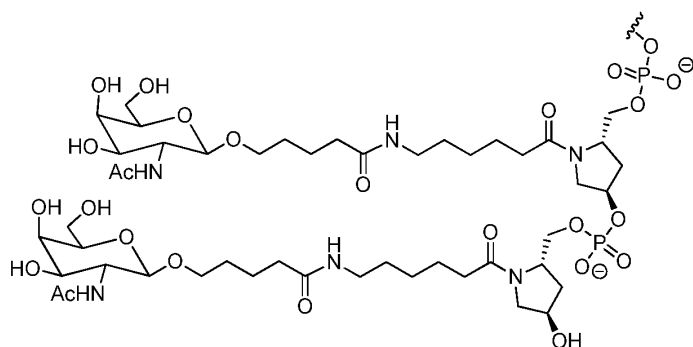
, wherein Y is O or S and n is 3-6 (Formula XXV);



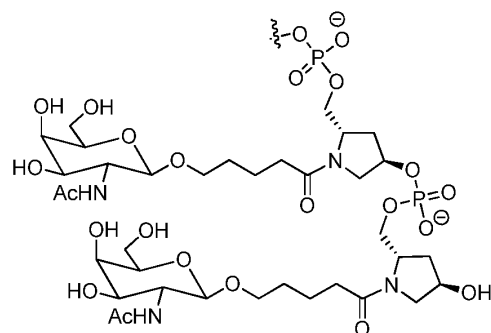
Formula XXVI;



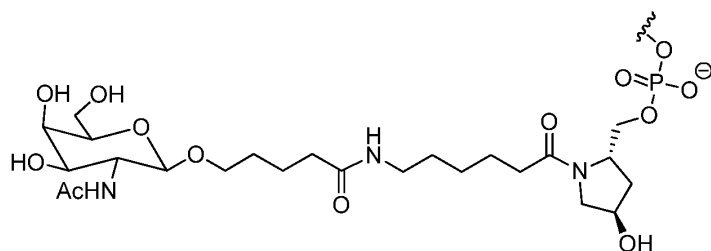
Formula XXVII; Formula XXIX;



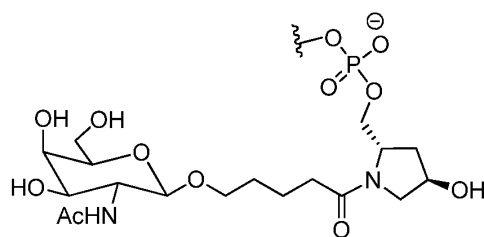
Formula XXXI;



Formula XXX;

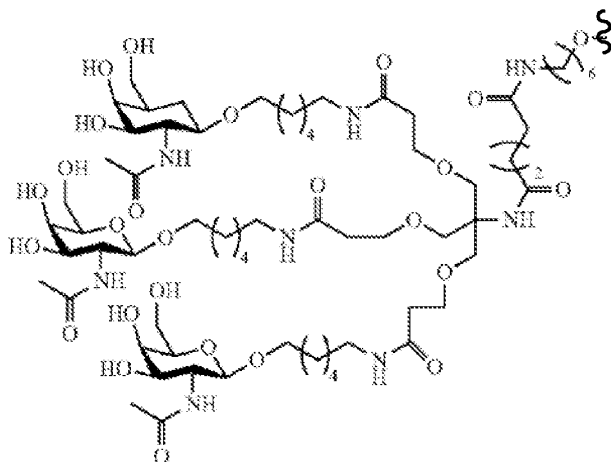


, and



Formula XXXII;

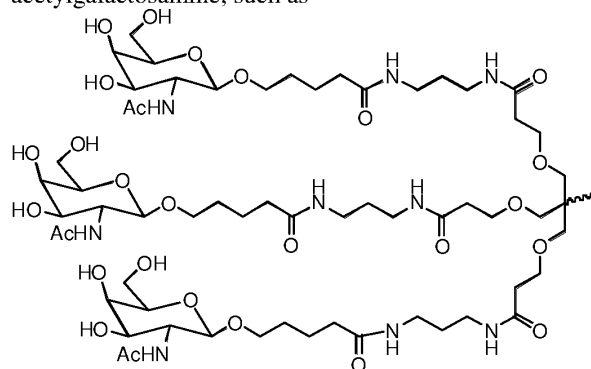
5 Formula XXXIII.



Formula XXXIV.

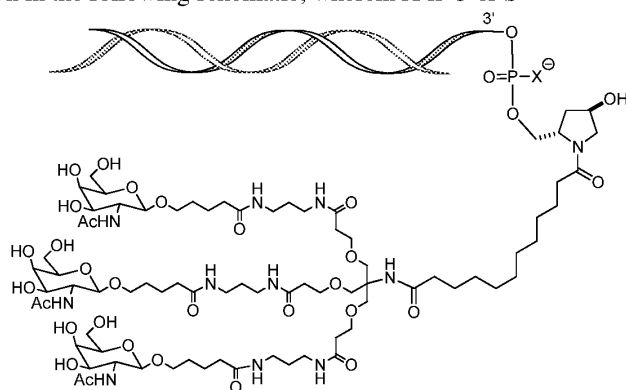
In another embodiment, a carbohydrate conjugate for use in the compositions and methods of the invention is a monosaccharide. In one embodiment, the monosaccharide is an N-

5 acetylgalactosamine, such as

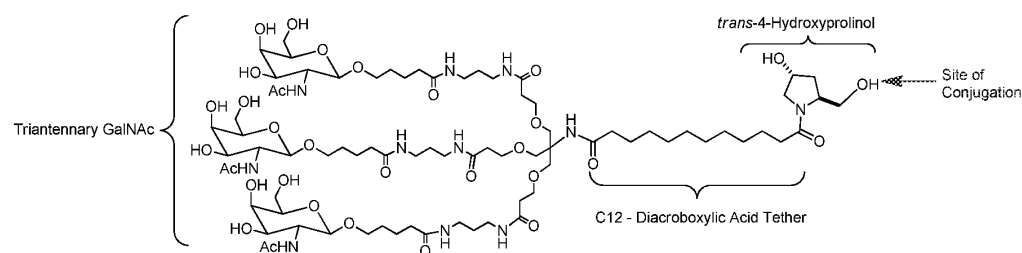


Formula II.

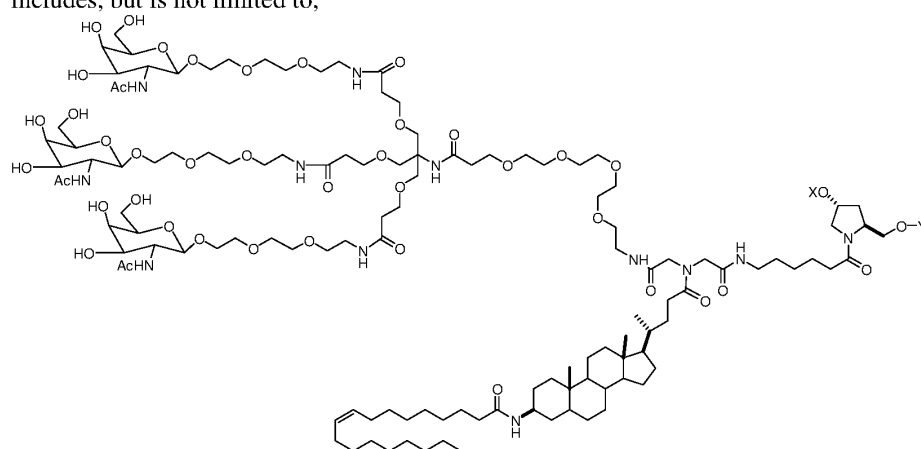
In some embodiments, the RNAi agent is attached to the carbohydrate conjugate *via* a linker as shown in the following schematic, wherein X is O or S



In some embodiments, the RNAi agent is conjugated to L96 as defined in Table 1 and shown below:



Another representative carbohydrate conjugate for use in the embodiments described herein includes, but is not limited to,

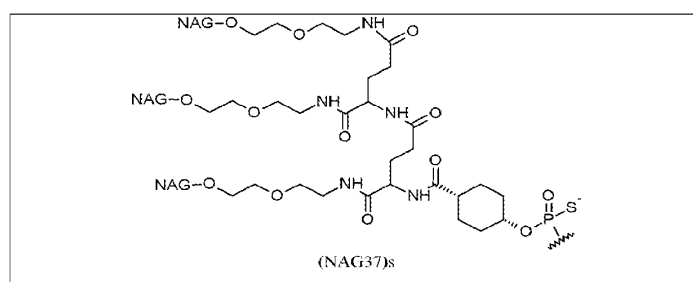


(Formula XXXVI), when one of X or Y is an oligonucleotide, the other is a hydrogen.

In some embodiments, a suitable ligand is a ligand disclosed in WO 2019/055633, the entire contents of which are incorporated herein by reference. In one embodiment the ligand comprises the structure below:

15

20



In certain embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a monovalent linker. In some embodiments, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a bivalent linker. In yet other

embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a trivalent linker.

In one embodiment, the double stranded RNAi agents of the invention comprise one or more GalNAc or GalNAc derivative attached to the iRNA agent. The GalNAc may be attached to any nucleotide *via* a linker on the sense strand or antisense strand. The GalNAc may be attached to the 5'-end of the sense strand, the 3' end of the sense strand, the 5'-end of the antisense strand, or the 3' – end of the antisense strand. In one embodiment, the GalNAc is attached to the 3' end of the sense strand, *e.g.*, *via* a trivalent linker.

In other embodiments, the double stranded RNAi agents of the invention comprise a plurality (e.g., 2, 3, 4, 5, or 6) GalNAc or GalNAc derivatives, each independently attached to a plurality of nucleotides of the double stranded RNAi agent through a plurality of linkers, *e.g.*, monovalent linkers.

In some embodiments, for example, when the two strands of an iRNA agent of the invention is part of one larger molecule connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming a hairpin loop comprising, a plurality of unpaired nucleotides, each unpaired nucleotide within the hairpin loop may independently comprise a GalNAc or GalNAc derivative attached *via* a monovalent linker.

In some embodiments, the carbohydrate conjugate further comprises one or more additional ligands as described above, such as, but not limited to, a PK modulator or a cell permeation peptide.

Additional carbohydrate conjugates and linkers suitable for use in the present invention include those described in PCT Publication Nos. WO 2014/179620 and WO 2014/179627, the entire contents of each of which are incorporated herein by reference.

D. Linkers

In some embodiments, the conjugate or ligand described herein can be attached to an iRNA oligonucleotide with various linkers that can be cleavable or non-cleavable.

The term "linker" or "linking group" means an organic moiety that connects two parts of a compound, *e.g.*, covalently attaches two parts of a compound. Linkers typically comprise a direct bond or an atom such as oxygen or sulfur, a unit such as NR₂, C(O), C(O)NH, SO, SO₂, SO₂NH or a chain of atoms, such as, but not limited to, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, arylalkyl, arylalkenyl, arylalkynyl, heteroarylalkyl, heteroarylalkenyl, heteroarylalkynyl, heterocyclalkyl, heterocyclalkenyl, heterocyclalkynyl, aryl, heteroaryl, heterocycl, cycloalkyl, cycloalkenyl, alkylarylalkyl, alkylarylalkenyl, alkylarylalkynyl, alkenylarylalkyl, alkenylarylalkenyl, alkenylarylalkynyl, alkynylarylalkyl, alkynylarylalkenyl, alkynylarylalkynyl, alkylheteroarylalkyl, alkylheteroarylalkenyl, alkylheteroarylalkynyl, alkenylheteroarylalkyl, alkenylheteroarylalkenyl, alkenylheteroarylalkynyl, alkynylheteroarylalkyl, alkynylheteroarylalkenyl, alkynylheteroarylalkynyl, alkylheterocyclalkyl, alkylheterocyclalkenyl, alkylheterocyclalkynyl, alkenylheterocyclalkyl, alkenylheterocyclalkenyl, alkenylheterocyclalkynyl, alkynylheterocyclalkyl, alkynylheterocyclalkenyl, alkynylheterocyclalkynyl, alkylaryl, alkenylaryl, alkynylaryl,

alkylheteroaryl, alkenylheteroaryl, alkynylheteroaryl, which one or more methylenes can be interrupted or terminated by O, S, S(O), SO₂, N(R₈), C(O), substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, or substituted or unsubstituted heterocyclic; where R₈ is hydrogen, acyl, aliphatic, or substituted aliphatic. In one embodiment, the linker is about 1-24 atoms, 2-24, 3-24, 4-24, 5-24, 6-24, 6-18, 7-18, 8-18, 7-17, 8-17, 6-16, 7-17, or 8-16 atoms.

A cleavable linking group is one which is sufficiently stable outside the cell, but which upon entry into a target cell is cleaved to release the two parts the linker is holding together. In a preferred embodiment, the cleavable linking group is cleaved at least about 10 times, 20, times, 30 times, 40 times, 50 times, 60 times, 70 times, 80 times, 90 times, or more, or at least 100 times faster in a target cell or under a first reference condition (which can, *e.g.*, be selected to mimic or represent intracellular conditions) than in the blood of a subject, or under a second reference condition (which can, *e.g.*, be selected to mimic or represent conditions found in the blood or serum).

Cleavable linking groups are susceptible to cleavage agents, *e.g.*, pH, redox potential, or the presence of degradative molecules. Generally, cleavage agents are more prevalent or found at higher levels or activities inside cells than in serum or blood. Examples of such degradative agents include: redox agents which are selected for particular substrates or which have no substrate specificity, including, *e.g.*, oxidative or reductive enzymes or reductive agents such as mercaptans, present in cells, that can degrade a redox cleavable linking group by reduction; esterases; endosomes or agents that can create an acidic environment, *e.g.*, those that result in a pH of five or lower; enzymes that can hydrolyze or degrade an acid cleavable linking group by acting as a general acid, peptidases (which can be substrate specific), and phosphatases.

A cleavable linkage group, such as a disulfide bond can be susceptible to pH. The pH of human serum is 7.4, while the average intracellular pH is slightly lower, ranging from about 7.1-7.3. Endosomes have a more acidic pH, in the range of 5.5-6.0, and lysosomes have an even more acidic pH at around 5.0. Some linkers will have a cleavable linking group that is cleaved at a preferred pH, thereby releasing a cationic lipid from the ligand inside the cell, or into the desired compartment of the cell.

A linker can include a cleavable linking group that is cleavable by a particular enzyme. The type of cleavable linking group incorporated into a linker can depend on the cell to be targeted. For example, a liver-targeting ligand can be linked to a cationic lipid through a linker that includes an ester group. Liver cells are rich in esterases, and therefore the linker will be cleaved more efficiently in liver cells than in cell types that are not esterase-rich. Other cell-types rich in esterases include cells of the lung, renal cortex, and testis.

Linkers that contain peptide bonds can be used when targeting cell types rich in peptidases, such as liver cells and synoviocytes.

In general, the suitability of a candidate cleavable linking group can be evaluated by testing the ability of a degradative agent (or condition) to cleave the candidate linking group. It will also be desirable to also test the candidate cleavable linking group for the ability to resist cleavage in the blood or when in contact with other non-target tissue. Thus, one can determine the relative

susceptibility to cleavage between a first and a second condition, where the first is selected to be indicative of cleavage in a target cell and the second is selected to be indicative of cleavage in other tissues or biological fluids, *e.g.*, blood or serum. The evaluations can be carried out in cell free systems, in cells, in cell culture, in organ or tissue culture, or in whole animals. It can be useful to make initial evaluations in cell-free or culture conditions and to confirm by further evaluations in whole animals. In preferred embodiments, useful candidate compounds are cleaved at least about 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 times faster in the cell (or under *in vitro* conditions selected to mimic intracellular conditions) as compared to blood or serum (or under *in vitro* conditions selected to mimic extracellular conditions).

i. Redox cleavable linking groups

In certain embodiments, a cleavable linking group is a redox cleavable linking group that is cleaved upon reduction or oxidation. An example of reductively cleavable linking group is a disulphide linking group (-S-S-). To determine if a candidate cleavable linking group is a suitable “reductively cleavable linking group,” or for example is suitable for use with a particular iRNA moiety and particular targeting agent one can look to methods described herein. For example, a candidate can be evaluated by incubation with dithiothreitol (DTT), or other reducing agent using reagents known in the art, which mimic the rate of cleavage which would be observed in a cell, *e.g.*, a target cell. The candidates can also be evaluated under conditions which are selected to mimic blood or serum conditions. In one, candidate compounds are cleaved by at most about 10% in the blood. In other embodiments, useful candidate compounds are degraded at least about 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, or about 100 times faster in the cell (or under *in vitro* conditions selected to mimic intracellular conditions) as compared to blood (or under *in vitro* conditions selected to mimic extracellular conditions). The rate of cleavage of candidate compounds can be determined using standard enzyme kinetics assays under conditions chosen to mimic intracellular media and compared to conditions chosen to mimic extracellular media.

ii. Phosphate-based cleavable linking groups

In other embodiments, a cleavable linker comprises a phosphate-based cleavable linking group. A phosphate-based cleavable linking group is cleaved by agents that degrade or hydrolyze the phosphate group. An example of an agent that cleaves phosphate groups in cells are enzymes such as phosphatases in cells. Examples of phosphate-based linking groups are -O-P(O)(ORk)-O-, -O-P(S)(ORk)-O-, -O-P(S)(SRk)-O-, -S-P(O)(ORk)-O-, -O-P(O)(ORk)-S-, -S-P(O)(ORk)-S-, -O-P(S)(ORk)-S-, -S-P(S)(ORk)-O-, -O-P(O)(Rk)-O-, -O-P(S)(Rk)-O-, -S-P(O)(Rk)-O-, -S-P(S)(Rk)-O-, -S-P(O)(Rk)-S-, -O-P(S)(Rk)-S-. Preferred embodiments are -O-P(O)(OH)-O-, -O-P(S)(OH)-O-, -O-P(S)(SH)-O-, -S-P(O)(OH)-O-, -O-P(O)(OH)-S-, -S-P(O)(OH)-S-, -O-P(S)(OH)-S-, -S-P(S)(OH)-O-, -O-P(O)(H)-O-, -O-P(S)(H)-O-, -S-P(O)(H)-O-, -S-P(S)(H)-O-, -S-P(O)(H)-S-, and -O-P(S)(H)-S-. A preferred embodiment is -O-P(O)(OH)-O-. These candidates can be evaluated using methods analogous to those described above.

iii. Acid cleavable linking groups

In other embodiments, a cleavable linker comprises an acid cleavable linking group. An acid cleavable linking group is a linking group that is cleaved under acidic conditions. In preferred embodiments acid cleavable linking groups are cleaved in an acidic environment with a pH of about 5 6.5 or lower (*e.g.*, about 6.0, 5.5, 5.0, or lower), or by agents such as enzymes that can act as a general acid. In a cell, specific low pH organelles, such as endosomes and lysosomes can provide a cleaving environment for acid cleavable linking groups. Examples of acid cleavable linking groups include but are not limited to hydrazones, esters, and esters of amino acids. Acid cleavable groups can have the general formula $-C=NN-$, $C(O)O-$, or $-OC(O)-$. A preferred embodiment is when the carbon attached to 10 the oxygen of the ester (the alkoxy group) is an aryl group, substituted alkyl group, or tertiary alkyl group such as dimethyl pentyl or t-butyl. These candidates can be evaluated using methods analogous to those described above.

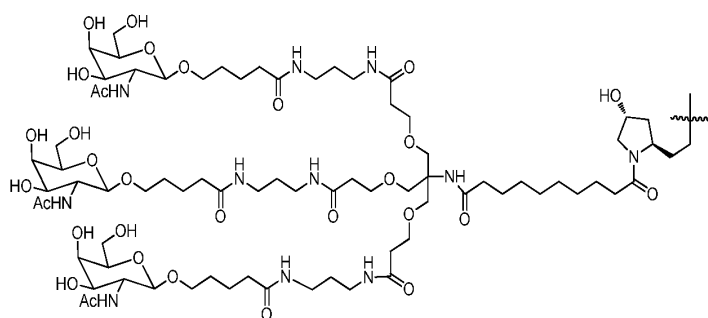
iv. Ester-based linking groups

15 In other embodiments, a cleavable linker comprises an ester-based cleavable linking group. An ester-based cleavable linking group is cleaved by enzymes such as esterases and amidases in cells. Examples of ester-based cleavable linking groups include, but are not limited to, esters of alkylene, alkenylene and alkynylene groups. Ester cleavable linking groups have the general formula $-C(O)O-$, or $-OC(O)-$. These candidates can be evaluated using methods analogous to those described above.

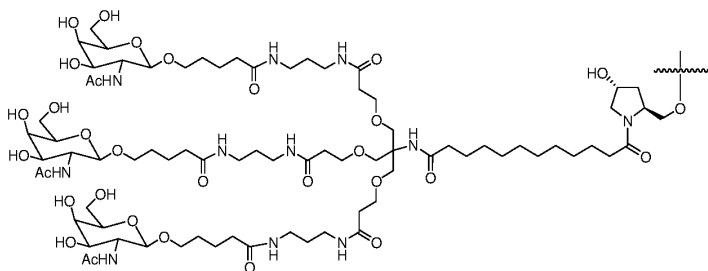
v. Peptide-based cleaving groups

In yet other embodiments, a cleavable linker comprises a peptide-based cleavable linking group. A peptide-based cleavable linking group is cleaved by enzymes such as peptidases and proteases in cells. Peptide-based cleavable linking groups are peptide bonds formed between amino 25 acids to yield oligopeptides (*e.g.*, dipeptides, tripeptides *etc.*) and polypeptides. Peptide-based cleavable groups do not include the amide group ($-C(O)NH-$). The amide group can be formed between any alkylene, alkenylene or alkynylene. A peptide bond is a special type of amide bond formed between amino acids to yield peptides and proteins. The peptide based cleavage group is generally limited to the peptide bond (*i.e.*, the amide bond) formed between amino acids yielding 30 peptides and proteins and does not include the entire amide functional group. Peptide-based cleavable linking groups have the general formula $-NHCH(R)A-C(O)NHCH(R)B-C(O)-$, where RA and RB are the R groups of the two adjacent amino acids. These candidates can be evaluated using methods analogous to those described above.

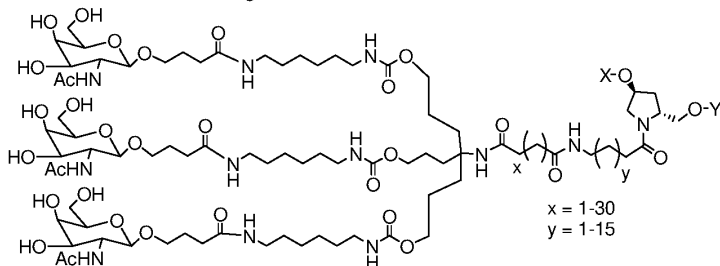
In some embodiments, an iRNA of the invention is conjugated to a carbohydrate through a 35 linker. Non-limiting examples of iRNA carbohydrate conjugates with linkers of the compositions and methods of the invention include, but are not limited to,



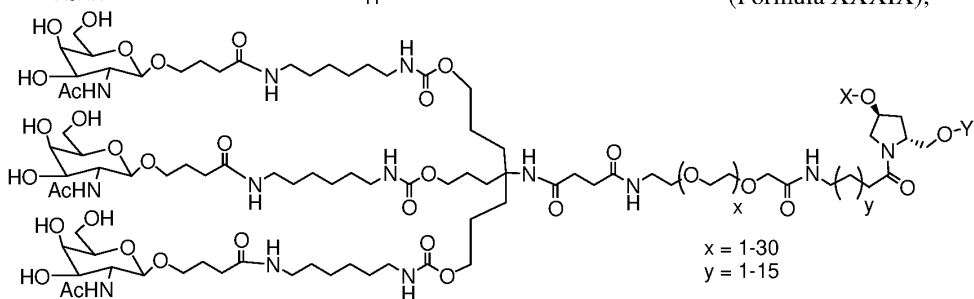
(Formula XXXVII),



(Formula XXXVIII),

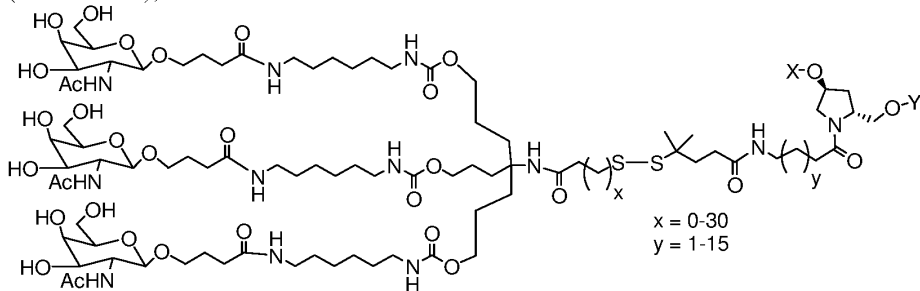


(Formula XXXIX),

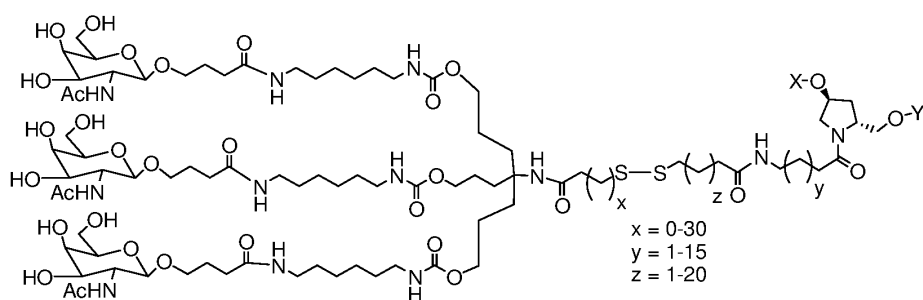


5

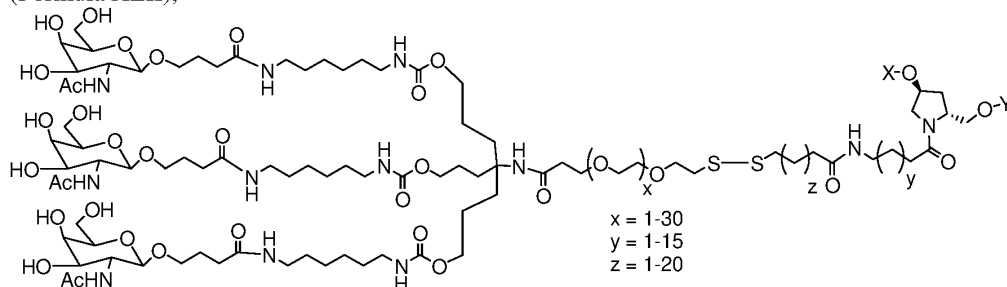
(Formula XL),



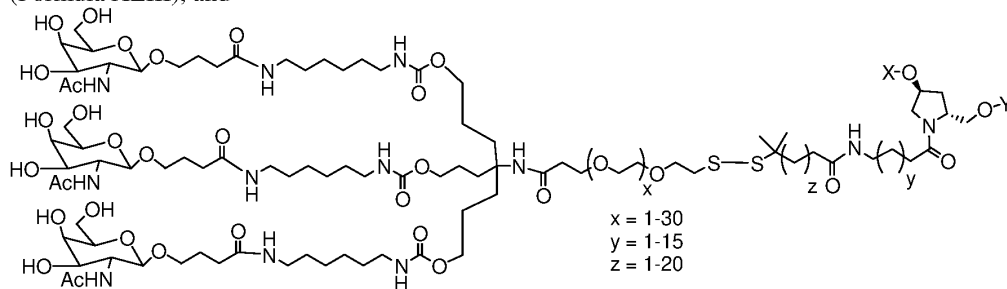
(Formula XLI),



(Formula XLII),



(Formula XLIII), and

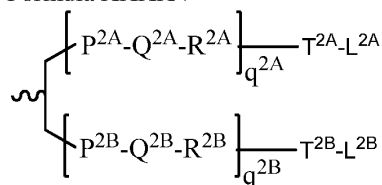


(Formula XLIV), when one of X or Y is an oligonucleotide, the other is a hydrogen.

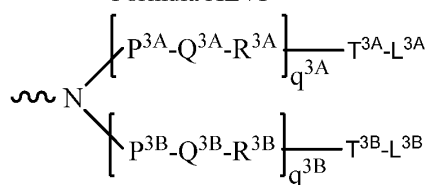
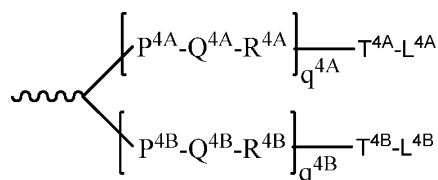
In certain embodiments of the compositions and methods of the invention, a ligand is one or more “GalNAc” (N-acetylgalactosamine) derivatives attached through a bivalent or trivalent branched linker.

10 In one embodiment, a dsRNA of the invention is conjugated to a bivalent or trivalent branched linker selected from the group of structures shown in any of formula (XLV) – (XLVI):

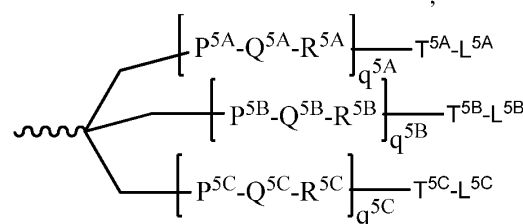
Formula XXXXV



Formula XLVI



Formula (VI)



Formula (VII)

Formula XLVII

Formula XLVIII

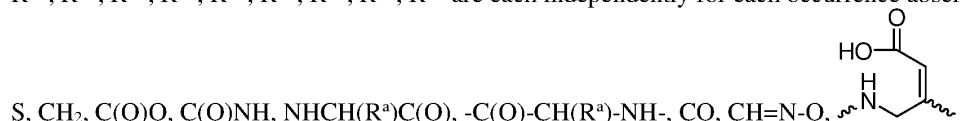
5 wherein:

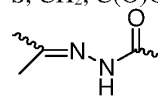
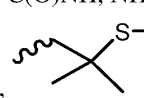
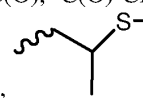
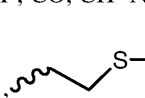
q^{2A}, q^{2B}, q^{3A}, q^{3B}, q^{4A}, q^{4B}, q^{5A}, q^{5B} and q^{5C} represent independently for each occurrence 0-20 and wherein the repeating unit can be the same or different;

P^{2A}, P^{2B}, P^{3A}, P^{3B}, P^{4A}, P^{4B}, P^{5A}, P^{5B}, P^{5C}, T^{2A}, T^{2B}, T^{3A}, T^{3B}, T^{4A}, T^{4B}, T^{4A}, T^{5B}, T^{5C} are each independently for each occurrence absent, CO, NH, O, S, OC(O), NHC(O), CH₂, CH₂NH or CH₂O;

10 Q^{2A}, Q^{2B}, Q^{3A}, Q^{3B}, Q^{4A}, Q^{4B}, Q^{5A}, Q^{5B}, Q^{5C} are independently for each occurrence absent, alkylene, substituted alkylene wherein one or more methylenes can be interrupted or terminated by one or more of O, S, S(O), SO₂, N(R^N), C(R')=C(R''), C≡C or C(O);

R^{2A}, R^{2B}, R^{3A}, R^{3B}, R^{4A}, R^{4B}, R^{5A}, R^{5B}, R^{5C} are each independently for each occurrence absent, NH, O,



15 , , , , or heterocyclyl;

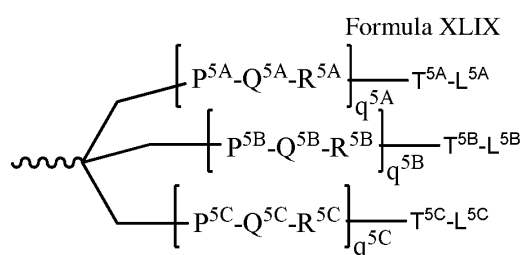
L^{2A}, L^{2B}, L^{3A}, L^{3B}, L^{4A}, L^{4B}, L^{5A}, L^{5B} and L^{5C} represent the ligand; *i.e.* each independently for each

occurrence a monosaccharide (such as GalNAc), disaccharide, trisaccharide, tetrasaccharide,

oligosaccharide, or polysaccharide; and R^a is H or amino acid side chain. Trivalent conjugating

GalNAc derivatives are particularly useful for use with RNAi agents for inhibiting the expression of a

20 target gene, such as those of formula (XLIX):



Formula (VII)

wherein L^{5A} , L^{5B} and L^{5C} represent a monosaccharide, such as GalNAc derivative.

Examples of suitable bivalent and trivalent branched linker groups conjugating GalNAc derivatives include, but are not limited to, the structures recited above as formulas II, VII, XI, X, and XIII.

Representative U.S. Patents that teach the preparation of RNA conjugates include, but are not limited to, U.S. Patent Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241; 5,391,723; 5,416,203; 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928; 5,688,941; 6,294,664; 6,320,017; 6,576,752; 6,783,931; 6,900,297; 7,037,646; and 8,106,022, the entire contents of each of which are hereby incorporated herein by reference.

It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the aforementioned modifications can be incorporated in a single compound or even at a single nucleoside within an iRNA. The present invention also includes iRNA compounds that are chimeric compounds.

“Chimeric” iRNA compounds or “chimeras,” in the context of this invention, are iRNA compounds, preferably dsRNAi agents, that contain two or more chemically distinct regions, each made up of at least one monomer unit, *i.e.*, a nucleotide in the case of a dsRNA compound. These iRNAs typically contain at least one region wherein the RNA is modified so as to confer upon the iRNA increased resistance to nuclease degradation, increased cellular uptake, or increased binding affinity for the target nucleic acid. An additional region of the iRNA can serve as a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of iRNA inhibition of gene expression. Consequently, comparable results can often be obtained with shorter iRNAs when chimeric dsRNAs are used, compared to phosphorothioate deoxy dsRNAs hybridizing to the same target region. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art.

In certain instances, the RNA of an iRNA can be modified by a non-ligand group. A number of non-ligand molecules have been conjugated to iRNAs in order to enhance the activity, cellular distribution or cellular uptake of the iRNA, and procedures for performing such conjugations are available in the scientific literature. Such non-ligand moieties have included lipid moieties, such as cholesterol (Kubo, T. *et al.*, *Biochem. Biophys. Res. Comm.*, 2007, 365(1):54-61; Letsinger *et al.*, *Proc. Natl. Acad. Sci. USA*, 1989, 86:6553), cholic acid (Manoharan *et al.*, *Bioorg. Med. Chem. Lett.*, 1994, 4:1053), a thioether, *e.g.*, hexyl-S-tritylthiol (Manoharan *et al.*, *Ann. N.Y. Acad. Sci.*, 1992, 660:306; Manoharan *et al.*, *Bioorg. Med. Chem. Lett.*, 1993, 3:2765), a thiocholesterol (Oberhauser *et al.*, *Nucl. Acids Res.*, 1992, 20:533), an aliphatic chain, *e.g.*, dodecandiol or undecyl residues (Saison-
 Behmoaras *et al.*, *EMBO J.*, 1991, 10:111; Kabanov *et al.*, *FEBS Lett.*, 1990, 259:327; Svinarchuk *et al.*, *Biochimie*, 1993, 75:49), a phospholipid, *e.g.*, di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan *et al.*, *Tetrahedron Lett.*, 1995, 36:3651; Shea *et al.*, *Nucl. Acids Res.*, 1990, 18:3777), a polyamine or a polyethylene glycol chain (Manoharan *et al.*, *Nucleosides & Nucleotides*, 1995, 14:969), or adamantane acetic acid (Manoharan *et al.*, *Tetrahedron Lett.*, 1995, 36:3651), a palmityl moiety (Mishra *et al.*, *Biochim. Biophys. Acta*, 1995, 1264:229), or an octadecylamine or hexylamino-carbonyl-oxysterol moiety (Crooke *et al.*, *J. Pharmacol. Exp. Ther.*, 1996, 277:923). Representative United States patents that teach the preparation of such RNA conjugates have been listed above. Typical conjugation protocols involve the synthesis of RNAs bearing an aminolinker at one or more positions of the sequence. The amino group is then reacted with the molecule being conjugated using appropriate coupling or activating reagents. The conjugation reaction can be performed either with the RNA still bound to the solid support or following cleavage of the RNA, in solution phase. Purification of the RNA conjugate by HPLC typically affords the pure conjugate.

IV. Delivery of an iRNA of the Invention

The delivery of an iRNA of the invention to a cell *e.g.*, a cell within a subject, such as a human subject (*e.g.*, a subject in need thereof, such as a subject susceptible to or diagnosed with an apolipoprotein C3-associated disorder, *e.g.*, hypertriglyceridemia) can be achieved in a number of different ways. For example, delivery may be performed by contacting a cell with an iRNA of the invention either *in vitro* or *in vivo*. *In vivo* delivery may also be performed directly by administering a composition comprising an iRNA, *e.g.*, a dsRNA, to a subject. Alternatively, *in vivo* delivery may be performed indirectly by administering one or more vectors that encode and direct the expression of the iRNA. These alternatives are discussed further below.

In general, any method of delivering a nucleic acid molecule (*in vitro* or *in vivo*) can be adapted for use with an iRNA of the invention (see *e.g.*, Akhtar S. and Julian RL. (1992) *Trends Cell Biol.* 2(5):139-144 and WO94/02595, which are incorporated herein by reference in their entireties). For *in vivo* delivery, factors to consider in order to deliver an iRNA molecule include, for example, biological stability of the delivered molecule, prevention of non-specific effects, and accumulation of the delivered molecule in the target tissue. RNA interference has also shown success with local

delivery to the CNS by direct injection (Dorn, G., *et al.* (2004) *Nucleic Acids* 32:e49; Tan, PH., *et al.* (2005) *Gene Ther.* 12:59-66; Makimura, H., *et al.* (2002) *BMC Neurosci.* 3:18; Shishkina, GT., *et al.* (2004) *Neuroscience* 129:521-528; Thakker, ER., *et al.* (2004) *Proc. Natl. Acad. Sci. U.S.A.*

101:17270-17275; Akaneya, Y., *et al.* (2005) *J. Neurophysiol.* 93:594-602). Modification of the RNA

5 or the pharmaceutical carrier can also permit targeting of the iRNA to the target tissue and avoid undesirable off-target effects. iRNA molecules can be modified by chemical conjugation to lipophilic groups such as cholesterol to enhance cellular uptake and prevent degradation. For example, an iRNA directed against ApoB conjugated to a lipophilic cholesterol moiety was injected systemically into mice and resulted in knockdown of apoB mRNA in both the liver and jejunum (Soutschek, J., *et al.*

10 (2004) *Nature* 432:173-178).

In an alternative embodiment, the iRNA can be delivered using drug delivery systems such as a nanoparticle, a dendrimer, a polymer, liposomes, or a cationic delivery system. Positively charged cationic delivery systems facilitate binding of an iRNA molecule (negatively charged) and also enhance interactions at the negatively charged cell membrane to permit efficient uptake of an iRNA

15 by the cell. Cationic lipids, dendrimers, or polymers can either be bound to an iRNA, or induced to form a vesicle or micelle (see *e.g.*, Kim SH, *et al.* (2008) *Journal of Controlled Release* 129(2):107-116) that encases an iRNA. The formation of vesicles or micelles further prevents degradation of the iRNA when administered systemically. Methods for making and administering cationic- iRNA complexes are well within the abilities of one skilled in the art (see *e.g.*, Sorensen, DR, *et al.* (2003) *J. Mol. Biol.* 327:761-766; Verma, UN, *et al.* (2003) *Clin. Cancer Res.* 9:1291-1300; Arnold, AS *et al.* (2007) *J. Hypertens.* 25:197-205, which are incorporated herein by reference in their entirety). Some non-limiting examples of drug delivery systems useful for systemic delivery of iRNAs include DOTAP (Sorensen, DR., *et al.* (2003), *supra*; Verma, UN, *et al.* (2003), *supra*), "solid nucleic acid lipid particles" (Zimmermann, TS, *et al.* (2006) *Nature* 441:111-114), cardiolipin (Chien, PY, *et al.*

25 (2005) *Cancer Gene Ther.* 12:321-328; Pal, A, *et al.* (2005) *Int J. Oncol.* 26:1087-1091), polyethyleneimine (Bonnet ME, *et al.* (2008) *Pharm. Res.* Aug 16 Epub ahead of print; Aigner, A. (2006) *J. Biomed. Biotechnol.* 71659), Arg-Gly-Asp (RGD) peptides (Liu, S. (2006) *Mol. Pharm.* 3:472-487), and polyamidoamines (Tomalia, DA, *et al.* (2007) *Biochem. Soc. Trans.* 35:61-67; Yoo, H., *et al.* (1999) *Pharm. Res.* 16:1799-1804). In some embodiments, an iRNA forms a complex with

30 cyclodextrin for systemic administration. Methods for administration and pharmaceutical compositions of iRNAs and cyclodextrins can be found in U.S. Patent No. 7,427,605, which is herein incorporated by reference in its entirety.

A. Vector encoded iRNAs of the Invention

35 iRNA targeting the apolipoprotein C3 gene can be expressed from transcription units inserted into DNA or RNA vectors (see, *e.g.*, Couture, A, *et al.*, *TIG.* (1996), 12:5-10; Skillern, A, *et al.*, International PCT Publication No. WO 00/22113, Conrad, International PCT Publication No. WO 00/22114, and Conrad, U.S. Patent No. 6,054,299). Expression can be transient (on the order of hours to weeks) or sustained (weeks to months or longer), depending upon the specific construct used and

the target tissue or cell type. These transgenes can be introduced as a linear construct, a circular plasmid, or a viral vector, which can be an integrating or non-integrating vector. The transgene can also be constructed to permit it to be inherited as an extrachromosomal plasmid (Gassmann, *et al.*, *Proc. Natl. Acad. Sci. USA* (1995) 92:1292).

- 5 Viral vector systems which can be utilized with the methods and compositions described herein include, but are not limited to, (a) adenovirus vectors; (b) retrovirus vectors, including but not limited to lentiviral vectors, moloney murine leukemia virus, *etc.*; (c) adeno- associated virus vectors; (d) herpes simplex virus vectors; (e) SV 40 vectors; (f) polyoma virus vectors; (g) papilloma virus vectors; (h) picornavirus vectors; (i) pox virus vectors such as an orthopox, *e.g.*, vaccinia virus vectors
10 or avipox, *e.g.* canary pox or fowl pox; and (j) a helper-dependent or gutless adenovirus. Replication-defective viruses can also be advantageous. Different vectors will or will not become incorporated into the cells' genome. The constructs can include viral sequences for transfection, if desired. Alternatively, the construct can be incorporated into vectors capable of episomal replication, *e.g.* EPV and EBV vectors. Constructs for the recombinant expression of an iRNA will generally require
15 regulatory elements, *e.g.*, promoters, enhancers, *etc.*, to ensure the expression of the iRNA in target cells. Other aspects to consider for vectors and constructs are known in the art.

V. Pharmaceutical Compositions of the Invention

- The present invention also includes pharmaceutical compositions and formulations which
20 include the iRNAs of the invention. In one embodiment, provided herein are pharmaceutical compositions containing an iRNA, as described herein, and a pharmaceutically acceptable carrier. The pharmaceutical compositions containing the iRNA are useful for preventing or treating an apolipoprotein C3-associated disorder, *e.g.*, hypertriglyceridemia. Such pharmaceutical compositions are formulated based on the mode of delivery. One example is compositions that are formulated for
25 systemic administration *via* parenteral delivery, *e.g.*, by subcutaneous (SC), intramuscular (IM), or intravenous (IV) delivery. The pharmaceutical compositions of the invention may be administered in dosages sufficient to inhibit expression of an apolipoprotein C3 gene.

In some embodiments, the pharmaceutical compositions of the invention are sterile. In another embodiment, the pharmaceutical compositions of the invention are pyrogen free.

- 30 The pharmaceutical compositions of the invention may be administered in dosages sufficient to inhibit expression of an apolipoprotein C3 gene. In general, a suitable dose of an iRNA of the invention will be in the range of about 0.001 to about 200.0 milligrams per kilogram body weight of the recipient per day, generally in the range of about 1 to 50 mg per kilogram body weight per day. Typically, a suitable dose of an iRNA of the invention will be in the range of about 0.1 mg/kg to
35 about 5.0 mg/kg, preferably about 0.3 mg/kg and about 3.0 mg/kg. A repeat-dose regimen may include administration of a therapeutic amount of iRNA on a regular basis, such as every month, once every 3-6 months, or once a year. In certain embodiments, the iRNA is administered about once per month to about once per six months.

After an initial treatment regimen, the treatments can be administered on a less frequent basis. Duration of treatment can be determined based on the severity of disease.

In other embodiments, a single dose of the pharmaceutical compositions can be long lasting, such that doses are administered at not more than 1, 2, 3, or 4 month intervals. In some embodiments of the invention, a single dose of the pharmaceutical compositions of the invention is administered about once per month. In other embodiments of the invention, a single dose of the pharmaceutical compositions of the invention is administered quarterly (*i.e.*, about every three months). In other embodiments of the invention, a single dose of the pharmaceutical compositions of the invention is administered twice per year (*i.e.*, about once every six months).

The skilled artisan will appreciate that certain factors can influence the dosage and timing required to effectively treat a subject, including but not limited to mutations present in the subject, previous treatments, the general health or age of the subject, and other diseases present. Moreover, treatment of a subject with a prophylactically or therapeutically effective amount, as appropriate, of a composition can include a single treatment or a series of treatments.

The iRNA can be delivered in a manner to target a particular tissue (*e.g.*, hepatocytes).

Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, and liposome-containing formulations. These compositions can be generated from a variety of components that include, but are not limited to, preformed liquids, self-emulsifying solids, and self-emulsifying semisolids. Formulations include those that target the liver.

The pharmaceutical formulations of the present invention, which can conveniently be presented in unit dosage form, can be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers.

A. Additional Formulations

i. Emulsions

The compositions of the present invention can be prepared and formulated as emulsions.

Emulsions are typically heterogeneous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1 μm in diameter (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., Volume 1, p. 245; Block in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 2, p. 335; Higuchi *et al.*, in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., 1985, p. 301). Emulsions are often biphasic systems comprising two immiscible liquid phases intimately mixed and dispersed

with each other. In general, emulsions can be of either the water-in-oil (w/o) or the oil-in-water (o/w) variety. When an aqueous phase is finely divided into and dispersed as minute droplets into a bulk oily phase, the resulting composition is called a water-in-oil (w/o) emulsion. Alternatively, when an oily phase is finely divided into and dispersed as minute droplets into a bulk aqueous phase, the resulting composition is called an oil-in-water (o/w) emulsion. Emulsions can contain additional components in addition to the dispersed phases, and the active drug which can be present as a solution either in the aqueous phase, oily phase or itself as a separate phase. Pharmaceutical excipients such as emulsifiers, stabilizers, dyes, and anti-oxidants can also be present in emulsions as needed.

Pharmaceutical emulsions can also be multiple emulsions that are comprised of more than two phases such as, for example, in the case of oil-in-water-in-oil (o/w/o) and water-in-oil-in-water (w/o/w) emulsions. Such complex formulations often provide certain advantages that simple binary emulsions do not. Multiple emulsions in which individual oil droplets of an o/w emulsion enclose small water droplets constitute a w/o/w emulsion. Likewise a system of oil droplets enclosed in globules of water stabilized in an oily continuous phase provides an o/w/o emulsion.

Emulsions are characterized by little or no thermodynamic stability. Often, the dispersed or discontinuous phase of the emulsion is well dispersed into the external or continuous phase and maintained in this form through the means of emulsifiers or the viscosity of the formulation. Other means of stabilizing emulsions entail the use of emulsifiers that can be incorporated into either phase of the emulsion. Emulsifiers can broadly be classified into four categories: synthetic surfactants, naturally occurring emulsifiers, absorption bases, and finely dispersed solids (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

Synthetic surfactants, also known as surface active agents, have found wide applicability in the formulation of emulsions and have been reviewed in the literature (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rieger, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), Marcel Dekker, Inc., New York, N.Y., 1988, volume 1, p. 199). Surfactants are typically amphiphilic and comprise a hydrophilic and a hydrophobic portion. The ratio of the hydrophilic to the hydrophobic nature of the surfactant has been termed the hydrophile/lipophile balance (HLB) and is a valuable tool in categorizing and selecting surfactants in the preparation of formulations. Surfactants can be classified into different classes based on the nature of the hydrophilic group: nonionic, anionic, cationic, and amphoteric (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rieger, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285).

A large variety of non-emulsifying materials are also included in emulsion formulations and contribute to the properties of emulsions. These include fats, oils, waxes, fatty acids, fatty alcohols, fatty esters, humectants, hydrophilic colloids, preservatives, and antioxidants (Block, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

The application of emulsion formulations *via* dermatological, oral, and parenteral routes, and methods for their manufacture have been reviewed in the literature (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

ii. Microemulsions

In one embodiment of the present invention, the compositions of iRNAs and nucleic acids are formulated as microemulsions. A microemulsion can be defined as a system of water, oil, and amphiphile which is a single optically isotropic and thermodynamically stable liquid solution (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245). Typically microemulsions are systems that are prepared by first dispersing an oil in an aqueous surfactant solution and then adding a sufficient amount of a fourth component, generally an intermediate chain-length alcohol to form a transparent system. Therefore, microemulsions have also been described as thermodynamically stable, isotropically clear dispersions of two immiscible liquids that are stabilized by interfacial films of surface-active molecules (Leung and Shah, in: Controlled Release of Drugs: Polymers and Aggregate Systems, Rosoff, M., Ed., 1989, VCH Publishers, New York, pages 185-215).

iii. Microparticles

An iRNA of the invention may be incorporated into a particle, *e.g.*, a microparticle. Microparticles can be produced by spray-drying, but may also be produced by other methods including lyophilization, evaporation, fluid bed drying, vacuum drying, or a combination of these techniques.

iv. Penetration Enhancers

In one embodiment, the present invention employs various penetration enhancers to effect the efficient delivery of nucleic acids, particularly iRNAs, to the skin of animals. Most drugs are present in solution in both ionized and nonionized forms. However, usually only lipid soluble or lipophilic drugs readily cross cell membranes. It has been discovered that even non-lipophilic drugs can cross cell membranes if the membrane to be crossed is treated with a penetration enhancer. In addition to

aiding the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also enhance the permeability of lipophilic drugs.

Penetration enhancers can be classified as belonging to one of five broad categories, *i.e.*, surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants (see *e.g.*,

- 5 Malmsten, M. Surfactants and polymers in drug delivery, Informa Health Care, New York, NY, 2002; Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p.92). Each of the above mentioned classes of penetration enhancers and their use in manufacture of pharmaceutical compositions and delivery of pharmaceutical agents are well known in the art.

10 *v. Excipients*

- In contrast to a carrier compound, a “pharmaceutical carrier” or “excipient” is a pharmaceutically acceptable solvent, suspending agent, or any other pharmacologically inert vehicle for delivering one or more nucleic acids to an animal. The excipient can be liquid or solid and is selected, with the planned manner of administration in mind, so as to provide for the desired bulk, consistency, etc., when combined with a nucleic acid and the other components of a given pharmaceutical composition. Such agent are well known in the art.

vi. Other Components

- The compositions of the present invention can additionally contain other adjunct components conventionally found in pharmaceutical compositions, at their art-established usage levels. Thus, for example, the compositions can contain additional, compatible, pharmaceutically-active materials such as, for example, antipruritics, astringents, local anesthetics or anti-inflammatory agents, or can contain additional materials useful in physically formulating various dosage forms of the compositions of the present invention, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. However, such materials, when added, should not unduly interfere with the biological activities of the components of the compositions of the present invention. The formulations can be sterilized and, if desired, mixed with auxiliary agents, *e.g.*, lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings, or aromatic substances, and the like which do not deleteriously interact with the nucleic acid(s) of the formulation.

Aqueous suspensions can contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol, or dextran. The suspension can also contain stabilizers.

- In some embodiments, pharmaceutical compositions featured in the invention include (a) one or more iRNA and (b) one or more agents which function by a non-iRNA mechanism and which are useful in treating an apolipoprotein C3-associated disorder, *e.g.*, hypertriglyceridemia.

Toxicity and prophylactic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose prophylactically effective in 50% of

the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds that exhibit high therapeutic indices are preferred.

5 The data obtained from cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of compositions featured herein in the invention lies generally within a range of circulating concentrations that include the ED50, preferably an ED80 or ED90, with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the methods featured in the invention, the prophylactically effective dose can be estimated initially from cell
10 culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range of the compound or, when appropriate, of the polypeptide product of a target sequence (*e.g.*, achieving a decreased concentration of the polypeptide) that includes the IC50 (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) or higher levels of inhibition as determined in cell culture. Such information can be used to more
15 accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

In addition to their administration, as discussed above, the iRNAs featured in the invention can be administered in combination with other known agents used for the prevention or treatment of an apolipoprotein C3-associated disorder, *e.g.*, hypertriglyceridemia. In any event, the administering
20 physician can adjust the amount and timing of iRNA administration on the basis of results observed using standard measures of efficacy known in the art or described herein.

VI. Methods For Inhibiting Apolipoprotein C3 Expression

The present invention also provides methods of inhibiting expression of an APOC3 gene in a
25 cell. The methods include contacting a cell with an RNAi agent, *e.g.*, double stranded RNA agent, in an amount effective to inhibit expression of APOC3 in the cell, thereby inhibiting expression of APOC3 in the cell.

Contacting of a cell with an iRNA, *e.g.*, a double stranded RNA agent, may be done *in vitro* or *in vivo*. Contacting a cell *in vivo* with the iRNA includes contacting a cell or group of cells within
30 a subject, *e.g.*, a human subject, with the iRNA. Combinations of *in vitro* and *in vivo* methods of contacting a cell are also possible. Contacting a cell may be direct or indirect, as discussed above. Furthermore, contacting a cell may be accomplished *via* a targeting ligand, including any ligand described herein or known in the art. In preferred embodiments, the targeting ligand is a carbohydrate moiety, *e.g.*, a GalNAc₃ ligand, or any other ligand that directs the RNAi agent to a site of interest.

35 The term “inhibiting,” as used herein, is used interchangeably with “reducing,” “silencing,” “downregulating,” “suppressing”, and other similar terms, and includes any level of inhibition.

The phrase “inhibiting expression of a apolipoprotein C3” is intended to refer to inhibition of expression of any apolipoprotein C3 gene (such as, *e.g.*, a mouse apolipoprotein C3 gene, a rat apolipoprotein C3 gene, a monkey apolipoprotein C3 gene, or a human apolipoprotein C3 gene) as

well as variants or mutants of an apolipoprotein C3 gene. Thus, the apolipoprotein C3 gene may be a wild-type apolipoprotein C3 gene, a mutant apolipoprotein C3 gene, or a transgenic apolipoprotein C3 gene in the context of a genetically manipulated cell, group of cells, or organism.

“Inhibiting expression of an apolipoprotein C3 gene” includes any level of inhibition of an apolipoprotein C3 gene, *e.g.*, at least partial suppression of the expression of an apolipoprotein C3 gene. The expression of the apolipoprotein C3 gene may be assessed based on the level, or the change in the level, of any variable associated with apolipoprotein C3 gene expression, *e.g.*, apolipoprotein C3 mRNA level or apolipoprotein C3 protein level. This level may be assessed in an individual cell or in a group of cells, including, for example, a sample derived from a subject. It is understood that apolipoprotein C3 is expressed predominantly in the liver, but also in the brain, gall bladder, heart, and kidney, and is present in circulation.

Inhibition may be assessed by a decrease in an absolute or relative level of one or more variables that are associated with apolipoprotein C3 expression compared with a control level. The control level may be any type of control level that is utilized in the art, *e.g.*, a pre-dose baseline level, or a level determined from a similar subject, cell, or sample that is untreated or treated with a control (such as, *e.g.*, buffer only control or inactive agent control).

In some embodiments of the methods of the invention, expression of an apolipoprotein C3 gene is inhibited by at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, or to below the level of detection of the assay. In preferred embodiments, expression of an apolipoprotein C3 gene is inhibited by at least 70%. It is further understood that inhibition of apolipoprotein C3 expression in certain tissues, *e.g.*, in liver, without a significant inhibition of expression in other tissues, *e.g.*, brain, may be desirable. In preferred embodiments, expression level is determined using the assay method provided in Example 2 with a 10 nM siRNA concentration in the appropriate species matched cell line.

In certain embodiments, inhibition of expression *in vivo* is determined by knockdown of the human gene in a rodent expressing the human gene, *e.g.*, an AAV-infected mouse expressing the human target gene (*i.e.*, apolipoprotein C3), *e.g.*, when administered as a single dose, *e.g.*, at 3 mg/kg at the nadir of RNA expression. Knockdown of expression of an endogenous gene in a model animal system can also be determined, *e.g.*, after administration of a single dose at, *e.g.*, 3 mg/kg at the nadir of RNA expression. Such systems are useful when the nucleic acid sequence of the human gene and the model animal gene are sufficiently close such that the human iRNA provides effective knockdown of the model animal gene. RNA expression in liver is determined using the PCR methods provided in Example 2.

Inhibition of the expression of an apolipoprotein C3 gene may be manifested by a reduction of the amount of mRNA expressed by a first cell or group of cells (such cells may be present, for example, in a sample derived from a subject) in which an apolipoprotein C3 gene is transcribed and which has or have been treated (*e.g.*, by contacting the cell or cells with an iRNA of the invention, or by administering an iRNA of the invention to a subject in which the cells are or were present) such that the expression of an apolipoprotein C3 gene is inhibited, as compared to a second cell or group of

cells substantially identical to the first cell or group of cells but which has not or have not been so treated (control cell(s) not treated with an iRNA or not treated with an iRNA targeted to the gene of interest). In preferred embodiments, the inhibition is assessed by the method provided in Example 2 using a 10nM siRNA concentration in the species matched cell line and expressing the level of mRNA in treated cells as a percentage of the level of mRNA in control cells, using the following formula:

$$\frac{(\text{mRNA in control cells}) - (\text{mRNA in treated cells})}{(\text{mRNA in control cells})} \bullet 100\%$$

In other embodiments, inhibition of the expression of an apolipoprotein C3 gene may be assessed in terms of a reduction of a parameter that is functionally linked to apolipoprotein C3 gene expression, *e.g.*, apolipoprotein C3 protein level in blood or serum from a subject. Apolipoprotein C3 gene silencing may be determined in any cell expressing apolipoprotein C3, either endogenous or heterologous from an expression construct, and by any assay known in the art.

Inhibition of the expression of an apolipoprotein C3 protein may be manifested by a reduction in the level of the apolipoprotein C3 protein that is expressed by a cell or group of cells or in a subject sample (*e.g.*, the level of protein in a blood sample derived from a subject). As explained above, for the assessment of mRNA suppression, the inhibition of protein expression levels in a treated cell or group of cells may similarly be expressed as a percentage of the level of protein in a control cell or group of cells, or the change in the level of protein in a subject sample, *e.g.*, blood or serum derived therefrom.

A control cell, a group of cells, or subject sample that may be used to assess the inhibition of the expression of an apolipoprotein C3 gene includes a cell, group of cells, or subject sample that has not yet been contacted with an RNAi agent of the invention. For example, the control cell, group of cells, or subject sample may be derived from an individual subject (*e.g.*, a human or animal subject) prior to treatment of the subject with an RNAi agent or an appropriately matched population control.

The level of apolipoprotein C3 mRNA that is expressed by a cell or group of cells may be determined using any method known in the art for assessing mRNA expression. In one embodiment, the level of expression of apolipoprotein C3 in a sample is determined by detecting a transcribed polynucleotide, or portion thereof, *e.g.*, mRNA of the apolipoprotein C3 gene. RNA may be extracted from cells using RNA extraction techniques including, for example, using acid phenol/guanidine isothiocyanate extraction (RNAzol B; Biogenesis), RNeasyTM RNA preparation kits (Qiagen®) or PAXgeneTM (PreAnalytixTM, Switzerland). Typical assay formats utilizing ribonucleic acid hybridization include nuclear run-on assays, RT-PCR, RNase protection assays, northern blotting, *in situ* hybridization, and microarray analysis.

In some embodiments, the level of expression of apolipoprotein C3 is determined using a nucleic acid probe. The term “probe”, as used herein, refers to any molecule that is capable of selectively binding to a specific apolipoprotein C3. Probes can be synthesized by one of skill in the art, or derived from appropriate biological preparations. Probes may be specifically designed to be

labeled. Examples of molecules that can be utilized as probes include, but are not limited to, RNA, DNA, proteins, antibodies, and organic molecules.

Isolated mRNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or northern analyses, polymerase chain reaction (PCR) analyses and probe arrays.

- 5 One method for the determination of mRNA levels involves contacting the isolated mRNA with a nucleic acid molecule (probe) that can hybridize to apolipoprotein C3 mRNA. In one embodiment, the mRNA is immobilized on a solid surface and contacted with a probe, for example by running the isolated mRNA on an agarose gel and transferring the mRNA from the gel to a membrane, such as nitrocellulose. In an alternative embodiment, the probe(s) are immobilized on a solid surface and the
- 10 mRNA is contacted with the probe(s), for example, in an Affymetrix® gene chip array. A skilled artisan can readily adapt known mRNA detection methods for use in determining the level of apolipoprotein C3 mRNA.

- An alternative method for determining the level of expression of apolipoprotein C3 in a sample involves the process of nucleic acid amplification or reverse transcriptase (to prepare cDNA)
- 15 of for example mRNA in the sample, *e.g.*, by RT-PCR (the experimental embodiment set forth in Mullis, 1987, U.S. Patent No. 4,683,202), ligase chain reaction (Barany (1991) *Proc. Natl. Acad. Sci. USA* 88:189-193), self sustained sequence replication (Guatelli *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi *et al.* (1988) *Bio/Technology* 6:1197), rolling circle
- 20 replication (Lizardi *et al.*, U.S. Patent No. 5,854,033) or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers. In particular aspects of the invention, the level of expression of APOC3 is determined by quantitative fluorogenic RT-PCR (*i.e.*, the TaqMan™
- 25 System). In preferred embodiments, expression level is determined by the method provided in Example 2 using, *e.g.*, a 10nM siRNA concentration, in the species matched cell line.

- The expression levels of apolipoprotein C3 mRNA may be monitored using a membrane blot (such as used in hybridization analysis such as northern, Southern, dot, and the like), or microwells, sample tubes, gels, beads or fibers (or any solid support comprising bound nucleic acids). See U.S.
- 30 Patent Nos. 5,770,722, 5,874,219, 5,744,305, 5,677,195 and 5,445,934, which are incorporated herein by reference. The determination of apolipoprotein C3 expression level may also comprise using nucleic acid probes in solution.

- In preferred embodiments, the level of mRNA expression is assessed using branched DNA (bDNA) assays or real time PCR (qPCR). The use of these methods is described and exemplified in
- 35 the Examples presented herein. In preferred embodiments, expression level is determined by the method provided in Example 2 using a 10nM siRNA concentration in the species matched cell line.

The level of APOC3 protein expression may be determined using any method known in the art for the measurement of protein levels. Such methods include, for example, electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer

chromatography (TLC), hyperdiffusion chromatography, fluid or gel precipitin reactions, absorption spectroscopy, a colorimetric assays, spectrophotometric assays, flow cytometry, immunodiffusion (single or double), immunoelectrophoresis, western blotting, radioimmunoassay (RIA), enzyme-linked immunosorbent assays (ELISAs), immunofluorescent assays, electrochemiluminescence assays, and the like.

In some embodiments, the efficacy of the methods of the invention are assessed by a decrease in C3 mRNA or protein level (*e.g.*, in a liver biopsy).

In some embodiments of the methods of the invention, the iRNA is administered to a subject such that the iRNA is delivered to a specific site within the subject. The inhibition of expression of apolipoprotein C3 may be assessed using measurements of the level or change in the level of apolipoprotein C3 mRNA or apolipoprotein C3 protein in a sample derived from fluid or tissue from the specific site within the subject (*e.g.*, liver or blood).

As used herein, the terms detecting or determining a level of an analyte are understood to mean performing the steps to determine if a material, *e.g.*, protein, RNA, is present. As used herein, methods of detecting or determining include detection or determination of an analyte level that is below the level of detection for the method used.

VII. Prophylactic and Treatment Methods of the Invention

The present invention also provides methods of using an iRNA of the invention or a composition containing an iRNA of the invention to inhibit expression of apolipoprotein C3, thereby preventing or treating an apolipoprotein C3-associated disorder, *e.g.*, hypertriglyceridemia, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, polycystic ovary syndrome, kidney disease, obesity, type 2 diabetes mellitus (insulin resistance); hypertension; cardiovascular disorders, *e.g.*, atherosclerosis; and pancreatitis, *e.g.*, acute pancreatitis. In the methods of the invention the cell may be contacted with the siRNA *in vitro* or *in vivo*, *i.e.*, the cell may be within a subject.

A cell suitable for treatment using the methods of the invention may be any cell that expresses an apolipoprotein C3 gene, *e.g.*, a liver cell, a brain cell, a gall bladder cell, a heart cell, or a kidney cell, but preferably a liver cell. A cell suitable for use in the methods of the invention may be a mammalian cell, *e.g.*, a primate cell (such as a human cell, including human cell in a chimeric non-human animal, or a non-human primate cell, *e.g.*, a monkey cell or a chimpanzee cell), or a non-primate cell. In certain embodiments, the cell is a human cell, *e.g.*, a human liver cell. In the methods of the invention, apolipoprotein C3 expression is inhibited in the cell by at least 50, 55, 60, 65, 70, 75, 80, 85, 90, or 95, or to a level below the level of detection of the assay.

The *in vivo* methods of the invention may include administering to a subject a composition containing an iRNA, where the iRNA includes a nucleotide sequence that is complementary to at least a part of an RNA transcript of the apolipoprotein C3 gene of the mammal to which the RNAi agent is to be administered. The composition can be administered by any means known in the art including, but not limited to oral, intraperitoneal, or parenteral routes, including intracranial (*e.g.*, intraventricular, intraparenchymal, and intrathecal), intravenous, intramuscular, subcutaneous,

transdermal, airway (aerosol), nasal, rectal, and topical (including buccal and sublingual) administration. In certain embodiments, the compositions are administered by intravenous infusion or injection. In certain embodiments, the compositions are administered by subcutaneous injection. In certain embodiments, the compositions are administered by intramuscular injection.

- 5 In one aspect, the present invention also provides methods for inhibiting the expression of an apolipoprotein C3 gene in a mammal. The methods include administering to the mammal a composition comprising a dsRNA that targets an apolipoprotein C3 gene in a cell of the mammal and maintaining the mammal for a time sufficient to obtain degradation of the mRNA transcript of the apolipoprotein C3 gene, thereby inhibiting expression of the apolipoprotein C3 gene in the cell.
- 10 Reduction in gene expression can be assessed by any methods known in the art and by methods, *e.g.* qRT-PCR, described herein, *e.g.*, in Example 2. Reduction in protein production can be assessed by any methods known in the art, *e.g.* ELISA. In certain embodiments, a puncture liver biopsy sample serves as the tissue material for monitoring the reduction in the apolipoprotein C3 gene or protein expression. In other embodiments, a blood sample serves as the subject sample for monitoring the
- 15 reduction in the apolipoprotein C3 protein expression.

- The present invention further provides methods of treatment in a subject in need thereof, *e.g.*, a subject diagnosed with an apolipoprotein C3-associated disorder, such as, hypertriglyceridemia, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, polycystic ovary syndrome, kidney disease, obesity, type 2 diabetes mellitus (insulin resistance); hypertension; cardiovascular disorders,
- 20 *e.g.*, arteriosclerosis; and pancreatitis, *e.g.*, acute pancreatitis. .

- The present invention further provides methods of prophylaxis in a subject in need thereof. The treatment methods of the invention include administering an iRNA of the invention to a subject, *e.g.*, a subject that would benefit from a reduction of apolipoprotein C3 expression, in a prophylactically effective amount of an iRNA targeting an apolipoprotein C3 gene or a
- 25 pharmaceutical composition comprising an iRNA targeting an apolipoprotein C3 gene.

- In one embodiment, an apolipoprotein C3-associated disease is selected from the group consisting of hypertriglyceridemia, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, polycystic ovary syndrome, kidney disease, obesity, type 2 diabetes mellitus (insulin resistance); hypertension; cardiovascular disorders, *e.g.*, arteriosclerosis; and pancreatitis, *e.g.*, acute pancreatitis.

- 30 In one embodiment, an APOC3-associated disease is hypertriglyceridemia, or a high triglyceride levels. The levels of triglycerides in a serum of a subject, *e.g.*, a human subject, that may be indicative of hypertriglyceridemia are described in Oh, R. C. *et al.*, (2007) *American Family Physician*, 75(9):1366-1371. Specifically, hypertriglyceridemia may be associated with “borderline-high serum triglyceride levels” (*i.e.*, 150 to 199 mg per dL or 1.70 to 2.25 mmol per L); “high serum triglyceride levels” (*i.e.*, 200 to 499 mg per dL or 2.26 to 5.64 mmol per L); or “very high triglyceride
- 35 levels” (*i.e.*, 500 mg per dL or higher (or 5.65 mmol per L or higher)

In one embodiment, an APOC3-associated disease is primary hypertriglyceridemia. “Primary triglyceridemia” results from environmental or genetic causes (*e.g.*, a result of no obvious underlying medical cause). Exemplary diseases characterized as primary hypertriglyceridemias include, but are

not limited to, familial chylomicronemia (hyperlipoproteinemia type I), primary mixed hyperlipidemia (type 5), familial hypertriglyceridemia (hyperlipoproteinemia type 4), familial combined hyperlipoproteinemia (type 2B) and familial dysbetalipoproteinemia (hyperlipoproteinemia type 3).

5 In another embodiment, an APOC3-associated disease is secondary hypertriglyceridemia. "Secondary triglyceridemia" is caused by, or be associated with, other underlying disorders and conditions. Such disorders and/or conditions include, *e.g.*, obesity, metabolic syndrome, diabetes, fatty liver, alcohol use, renal disease, pregnancy, nonalcoholic fatty liver disorder, hypothyroidism, paraproteinemias (such as hypergammaglobulinemia in macroglobulinemia, myeloma, lymphoma and
10 lymphocytic leukemias), autoimmune disorders (such as systemic lupus erythematosus), intake of medications (such as antiretroviral drugs, including ritonavir and lopinavir, and antipsychotic medications, including clozapine and olanzapine), see G. Yuan *et al.*, (2007) *Canadian Medical Association Journal*, 176(8):1113-1120.

Any disorder that may be a cause of hypertriglyceridemia (*e.g.*, secondary
15 hypertriglyceridemia) or that may be a consequence of hypertriglyceridemia (*e.g.*, primary or secondary hypertriglyceridemia) is encompassed by the term "APOC3-associated disease". Non-limiting examples of APOC3-associated diseases include metabolic disorders, *e.g.*, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, polycystic ovary syndrome, kidney disease, obesity, type 2 diabetes mellitus (insulin resistance); hypertension; cardiovascular disorders, *e.g.*, arteriosclerosis;
20 and pancreatitis, *e.g.*, acute pancreatitis.

An iRNA of the invention may be administered as a "free iRNA." A free iRNA is administered in the absence of a pharmaceutical composition. The naked iRNA may be in a suitable buffer solution. The buffer solution may comprise acetate, citrate, prolamine, carbonate, or phosphate, or any combination thereof. In one embodiment, the buffer solution is phosphate buffered
25 saline (PBS). The pH and osmolarity of the buffer solution containing the iRNA can be adjusted such that it is suitable for administering to a subject.

Alternatively, an iRNA of the invention may be administered as a pharmaceutical composition, such as a dsRNA liposomal formulation.

Subjects that would benefit from an inhibition of APOC3 gene expression are subjects
30 susceptible to or diagnosed with an APOC3-associated disorder, such as hypertriglyceridemia, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, polycystic ovary syndrome, kidney disease, obesity, type 2 diabetes mellitus (insulin resistance); hypertension; cardiovascular disorders, *e.g.*, arteriosclerosis; and pancreatitis, *e.g.*, acute pancreatitis.

In an embodiment, the method includes administering a composition featured herein such that
35 expression of the target apolipoprotein C3 gene is decreased, such as for about 1, 2, 3, 4, 5, 6, 1-6, 1-3, or 3-6 months per dose. In certain embodiments, the composition is administered once every 3-6 months.

Preferably, the iRNAs useful for the methods and compositions featured herein specifically target RNAs (primary or processed) of the target apolipoprotein C3 gene. Compositions and methods

for inhibiting the expression of these genes using iRNAs can be prepared and performed as described herein.

Administration of the iRNA according to the methods of the invention may result prevention or treatment of an apolipoprotein C3-associated disorder, *e.g.*, hypertriglyceridemia, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, polycystic ovary syndrome, kidney disease, obesity, type 2 diabetes mellitus (insulin resistance); hypertension; cardiovascular disorders, *e.g.*, atherosclerosis; and pancreatitis, *e.g.*, acute pancreatitis.

Subjects can be administered a therapeutic amount of iRNA, such as about 0.01 mg/kg to about 200 mg/kg.

The iRNA is preferably administered subcutaneously, *i.e.*, by subcutaneous injection. One or more injections may be used to deliver the desired dose of iRNA to a subject. The injections may be repeated over a period of time.

The administration may be repeated on a regular basis. In certain embodiments, after an initial treatment regimen, the treatments can be administered on a less frequent basis. A repeat-dose regimen may include administration of a therapeutic amount of iRNA on a regular basis, such as once per month to once a year. In certain embodiments, the iRNA is administered about once per month to about once every three months, or about once every three months to about once every six months.

The invention further provides methods and uses of an iRNA agent or a pharmaceutical composition thereof for treating a subject that would benefit from reduction and/or inhibition of APOC3 gene expression, *e.g.*, a subject having an APOC3-associated disease, in combination with other pharmaceuticals and/or other therapeutic methods, *e.g.*, with known pharmaceuticals and/or known therapeutic methods, such as, for example, those which are currently employed for treating these disorders.

Accordingly, in some aspects of the invention, the methods which include either a single iRNA agent of the invention, further include administering to the subject one or more additional therapeutic agents.

The iRNA agent and an additional therapeutic agent and/or treatment may be administered at the same time and/or in the same combination, *e.g.*, parenterally, or the additional therapeutic agent can be administered as part of a separate composition or at separate times and/or by another method known in the art or described herein.

Examples of additional therapeutic agents include those known to treat hypertriglyceridemia and other diseases that are caused by, associated with or are a consequence of hypertriglyceridemia. For example, an iRNA featured in the invention can be administered with, *e.g.*, a PCSK9 inhibitor (*e.g.*, an anti-PCSK9 monoclonal antibody, *e.g.*, evolocumab (Repatha®) and alirocumab (Praluent®), a dsRNA agent targeting PCSK9 (*e.g.*, Inclisran)), an HMG-CoA reductase inhibitor (*e.g.*, a statin), a fibrate, a bile acid sequestrant, niacin, an antiplatelet agent, an angiotensin converting enzyme inhibitor, an angiotensin II receptor antagonist (*e.g.*, losartan potassium, such as Merck & Co. 's Cozaar®), an acylCoA cholesterol acetyltransferase (ACAT) inhibitor, a cholesterol absorption inhibitor, a cholesterol ester transfer protein (CETP) inhibitor, a microsomal triglyceride transfer

- protein (MTTP) inhibitor, a cholesterol modulator, a bile acid modulator, a peroxisome proliferation activated receptor (PPAR) agonist, a gene-based therapy, a composite vascular protectant (*e.g.*, AGI-1067, from Atherogenics), a glycoprotein IIb/IIIa inhibitor, aspirin or an aspirin-like compound, an IBAT inhibitor (*e.g.*, S-8921, from Shionogi), a squalene synthase inhibitor, a monocyte
- 5 chemoattractant protein (MCP)-I inhibitor, or fish oil. Exemplary HMG-CoA reductase inhibitors include atorvastatin (Pfizer's Lipitor®/Tahor/Sortis/Torvast/Cardyl), pravastatin (Bristol-Myers Squibb's Pravachol, Sankyo's Mevalotin/Sanapray), simvastatin (Merck's Zocor®/Sinvacor, Boehringer Ingelheim's Denan, Banyu's Lipovas), lovastatin (Merck's Mevacor/Mevinacor, Bexal's Lovastatina, Cepa; Schwarz Pharma's Liposcler), fluvastatin (Novartis' Lescol®/Locol/Lochol,
- 10 Fujisawa's Cranoc, Solvay's Digaril), cerivastatin (Bayer's Lipobay/GlaxoSmithKline's Baycol), rosuvastatin (AstraZeneca's Crestor®), and pitivastatin (itavastatin/risivastatin) (Nissan Chemical, Kowa Kogyo, Sankyo, and Novartis). Exemplary fibrates include, *e.g.*, bezafibrate (*e.g.*, Roche's Befizal®/Cedur®/Bezalip®, Kissei's Bezatol), clofibrate (*e.g.*, Wyeth's Atromid-S®), fenofibrate (*e.g.*, Fournier's Lipidil/Lipantil, Abbott's Tricor®, Takeda's Lipantil, generics), gemfibrozil (*e.g.*,
- 15 Pfizer's Lopid/Lipur) and ciprofibrate (Sanofi-Synthelabo's Modalim®). Exemplary bile acid sequestrants include, *e.g.*, cholestyramine (Bristol-Myers Squibb's Questran® and Questran Light™), colestipol (*e.g.*, Pharmacia's Colestid), and colestevlam (Genzyme/Sankyo's WelChol™). Exemplary niacin therapies include, *e.g.*, immediate release formulations, such as Aventis' Nicobid, Upsher-Smith's Niacor, Aventis' Nicolac, and Sanwakagaku's Perycit. Niacin extended release formulations
- 20 include, *e.g.*, Kos Pharmaceuticals' Niaspan and Upsher-Smith's SIO- Niacin. Exemplary antiplatelet agents include, *e.g.*, aspirin (*e.g.*, Bayer's aspirin), clopidogrel (Sanofi-Synthelabo/Bristol-Myers Squibb's Plavix), and ticlopidine (*e.g.*, Sanofi-Synthelabo's Ticlid and Daiichi's Panalidine). Other aspirin-like compounds useful in combination with a dsRNA targeting APOC3 include, *e.g.*, Asacard (slow-release aspirin, by Pharmacia) and Pamicoqrel (Kanebo/Angelini Ricerche/CEPA). Exemplary
- 25 angiotensin-converting enzyme inhibitors include, *e.g.*, ramipril (*e.g.*, Aventis' Altace) and enalapril (*e.g.*, Merck & Co.'s Vasotec). Exemplary acyl CoA cholesterol acetyltransferase (ACAT) inhibitors include, *e.g.*, avasimibe (Pfizer), eflucimibe (BioMérieux Pierre Fabre/Eli Lilly), CS-505 (Sankyo and Kyoto), and SMP-797 (Sumitomo). Exemplary cholesterol absorption inhibitors include, *e.g.*, ezetimibe (Merck/Schering-Plough Pharmaceuticals Zetia®) and Pamaqueside (Pfizer). Exemplary CETP
- 30 inhibitors include, *e.g.*, Torcetrapib (also called CP-529414, Pfizer), JTT-705 (Japan Tobacco), and CETi-I (Avant Immunotherapeutics). Exemplary microsomal triglyceride transfer protein (MTTP) inhibitors include, *e.g.*, implitapide (Bayer), R-103757 (Janssen), and CP-346086 (Pfizer). Other exemplary cholesterol modulators include, *e.g.*, NO-1886 (Otsuka/TAP Pharmaceutical), CI-1027 (Pfizer), and WAY-135433 (Wyeth-Ayerst).
- 35 Exemplary bile acid modulators include, *e.g.*, HBS-107 (Hisamitsu/Banyu), Btg-511 (British Technology Group), BARI-1453 (Aventis), S-8921 (Shionogi), SD-5613 (Pfizer), and AZD-7806 (AstraZeneca). Exemplary peroxisome proliferation activated receptor (PPAR) agonists include, *e.g.*, tesaglitazar (AZ-242) (AstraZeneca), Netoglitazone (MCC-555) (Mitsubishi/Johnson & Johnson), GW-409544 (Ligand Pharmaceuticals/GlaxoSmithKline), GW-501516 (Ligand

Pharmaceuticals/GlaxoSmithKline), LY-929 (Ligand Pharmaceuticals and Eli Lilly), LY-465608 (Ligand Pharmaceuticals and Eli Lilly), LY-518674 (Ligand Pharmaceuticals and Eli Lilly), and MK-767 (Merck and Kyorin). Exemplary gene-based therapies include, *e.g.*, AdGWEGF 121.10 (GenVec), ApoA1 (UCB Pharma/Groupe Fournier), EG-004 (Trinam) (Ark Therapeutics), and ATP -

5 binding cassette transporter- A1 (ABCA1) (CV Therapeutics/Incyte, Aventis, Xenon). Exemplary Glycoprotein IIb/IIIa inhibitors include, *e.g.*, roxifiban (also called DMP754, Bristol-Myers Squibb), Gantofiban (Merck KGaA/Yamanouchi), and Cromafiban (Millennium Pharmaceuticals). Exemplary squalene synthase inhibitors include, *e.g.*, BMS- 1884941 (Bristol-Myers Squibb), CP-210172 (Pfizer), CP-295697 (Pfizer), CP-294838 (Pfizer), and TAK-475 (Takeda). An exemplary MCP-I

10 inhibitor is, *e.g.*, RS-504393 (Roche Bioscience). The anti-atherosclerotic agent BO- 653 (Chugai Pharmaceuticals), and the nicotinic acid derivative Nyclin (Yamanouchi Pharmaceuticals) are also appropriate for administering in combination with a dsRNA featured in the invention. Exemplary combination therapies suitable for administration with a dsRNA targeting APOC3 include, *e.g.*,

15 advicor (Niacin/lovastatin from Kos Pharmaceuticals), amlodipine/atorvastatin (Pfizer), and ezetimibe/simvastatin (*e.g.*, Vytorin® 10/10, 10/20, 10/40, and 10/80 tablets by Merck/Schering-Plough Pharmaceuticals). Agents for treating hypertriglyceridemia, and suitable for administration in combination with a dsRNA targeting APOC3 include, *e.g.*, lovastatin, niacin Altoprev® Extended-Release Tablets (Andrx Labs), lovastatin Caduet® Tablets (Pfizer), amlodipine besylate, atorvastatin calcium Crestor® Tablets (AstraZeneca), rosuvastatin calcium Lescol® Capsules (Novartis),

20 fluvastatin sodium Lescol® (Reliant, Novartis), fluvastatin sodium Lipitor® Tablets (Parke-Davis), atorvastatin calcium Lofibra® Capsules (Gate), Niaspan Extended-Release Tablets (Kos), niacin Pravachol Tablets (Bristol-Myers Squibb), pravastatin sodium TriCor® Tablets (Abbott), fenofibrate Vytorin® 10/10 Tablets (Merck/Schering-Plough Pharmaceuticals), ezetimibe, simvastatin WelChol™ Tablets (Sankyo), colesvelam hydrochloride Zetia® Tablets (Schering), ezetimibe

25 Zetia® Tablets (Merck/Schering-Plough Pharmaceuticals), and ezetimibe Zocor® Tablets (Merck).

In one embodiment, an iRNA agent is administered in combination with a PCSK9 inhibitor. In one embodiment, the PCSK9 inhibitor is an anti-PCSK9 monoclonal antibody, *e.g.*, evolocumab (Repatha®) and alirocumab (Praluent®). In another embodiment, the PCSK9 inhibitor is a dsRNA agent targeting PCSK9, *e.g.*, Inclisiran. In one embodiment, the iRNA agent is administered to the

30 patient, and then the additional therapeutic agent is administered to the patient (or vice versa). In another embodiment, the iRNA agent and the additional therapeutic agent are administered at the same time.

In one embodiment, an iRNA agent is administered in combination with ezetimibe/simvastatin combination (*e.g.*, Vytorin® (Merck/Schering-Plough Pharmaceuticals)). In

35 one embodiment, the iRNA agent is administered to the patient, and then the additional therapeutic agent is administered to the patient (or vice versa). In another embodiment, the iRNA agent and the additional therapeutic agent are administered at the same time.

The iRNA agent and an additional therapeutic agent and/or treatment may be administered at the same time and/or in the same combination, *e.g.*, parenterally, or the additional therapeutic agent

can be administered as part of a separate composition or at separate times and/or by another method known in the art or described herein.

VIII. Kits

- 5 In certain aspects, the instant disclosure provides kits that include a suitable container containing a pharmaceutical formulation of a siRNA compound, *e.g.*, a double-stranded siRNA compound, or ssiRNA compound, (*e.g.*, a precursor, *e.g.*, a larger siRNA compound which can be processed into a ssiRNA compound, or a DNA which encodes an siRNA compound, *e.g.*, a double-stranded siRNA compound, or ssiRNA compound, or precursor thereof).
- 10 Such kits include one or more dsRNA agent(s) and instructions for use, *e.g.*, instructions for administering a prophylactically or therapeutically effective amount of a dsRNA agent(s). The dsRNA agent may be in a vial or a pre-filled syringe. The kits may optionally further comprise means for administering the dsRNA agent (*e.g.*, an injection device, such as a pre-filled syringe), or means for measuring the inhibition of APOC3 (*e.g.*, means for measuring the inhibition of APOC3 mRNA,
- 15 APOC3 protein, and/or APOC3 activity). Such means for measuring the inhibition of APOC3 may comprise a means for obtaining a sample from a subject, such as, *e.g.*, a plasma sample. The kits of the invention may optionally further comprise means for determining the therapeutically effective or prophylactically effective amount.

- In certain embodiments the individual components of the pharmaceutical formulation may be
- 20 provided in one container, *e.g.*, a vial or a pre-filled syringe. Alternatively, it may be desirable to provide the components of the pharmaceutical formulation separately in two or more containers, *e.g.*, one container for a siRNA compound preparation, and at least another for a carrier compound. The kit may be packaged in a number of different configurations such as one or more containers in a single box. The different components can be combined, *e.g.*, according to instructions provided with the kit.
- 25 The components can be combined according to a method described herein, *e.g.*, to prepare and administer a pharmaceutical composition. The kit can also include a delivery device.

- This invention is further illustrated by the following examples which should not be construed as limiting. The entire contents of all references, patents and published patent applications cited
- 30 throughout this application, as well as the informal Sequence Listing and Figures, are hereby incorporated herein by reference.

35

EXAMPLES

Example 1. iRNA Synthesis

Source of reagents

Where the source of a reagent is not specifically given herein, such reagent can be obtained
5 from any supplier of reagents for molecular biology at a quality/purity standard for application in molecular biology.

siRNA Design

siRNAs targeting the human Apolipoprotein C3 (APOC3) gene (human: NCBI refseqID
10 NM_000040.3; NCBI GeneID: 345) were designed using custom R and Python scripts. The human NM_000040.3 REFSEQ mRNA, has a length of 535 bases.

Detailed lists of the unmodified APOC3 sense and antisense strand nucleotide sequences are shown in Tables 2 and 4. Detailed lists of the modified apolipoprotein C3 sense and antisense strand nucleotide sequences are shown in Tables 3 and 5.

15 It is to be understood that, throughout the application, a duplex name without a decimal is equivalent to a duplex name with a decimal which merely references the batch number of the duplex. For example, AD-959917 is equivalent to AD-959917.1.

siRNA Synthesis

20 siRNAs were synthesized and annealed using routine methods known in the art.

Briefly, siRNA sequences were synthesized on a 1 μ mol scale using a Mermade 192 synthesizer (BioAutomation) with phosphoramidite chemistry on solid supports. The solid support was controlled pore glass (500-1000 Å) loaded with a custom GalNAc ligand (3'-GalNAc conjugates), universal solid support (AM Chemicals), or the first nucleotide of interest. Ancillary
25 synthesis reagents and standard 2-cyanoethyl phosphoramidite monomers (2'-deoxy-2'-fluoro, 2'-O-methyl, RNA, DNA) were obtained from Thermo-Fisher (Milwaukee, WI), Hongene (China), or Chemgenes (Wilmington, MA, USA). Additional phosphoramidite monomers were procured from commercial suppliers, prepared in-house, or procured using custom synthesis from various CMOs. Phosphoramidites were prepared at a concentration of 100 mM in either acetonitrile or 9:1
30 acetonitrile:DMF and were coupled using 5-Ethylthio-1H-tetrazole (ETT, 0.25 M in acetonitrile) with a reaction time of 400 s. Phosphorothioate linkages were generated using a 100 mM solution of 3-((Dimethylamino-methylidene) amino)-3H-1,2,4-dithiazole-3-thione (DDTT, obtained from Chemgenes (Wilmington, MA, USA)) in anhydrous acetonitrile/pyridine (9:1 v/v). Oxidation time was 5 minutes. All sequences were synthesized with final removal of the DMT group ("DMT-Off").

35 Upon completion of the solid phase synthesis, solid-supported oligoribonucleotides were treated with 300 μ L of Methylamine (40% aqueous) at room temperature in 96 well plates for approximately 2 hours to afford cleavage from the solid support and subsequent removal of all additional base-labile protecting groups. For sequences containing any natural ribonucleotide linkages (2'-OH) protected with a tert-butyl dimethyl silyl (TBDMS) group, a second deprotection step was

performed using TEA.3HF (triethylamine trihydrofluoride). To each oligonucleotide solution in aqueous methylamine was added 200 μ L of dimethyl sulfoxide (DMSO) and 300 μ L TEA.3HF and the solution was incubated for approximately 30 mins at 60 °C. After incubation, the plate was allowed to come to room temperature and crude oligonucleotides were precipitated by the addition of 1 mL of 9:1 acetonitrile:ethanol or 1:1 ethanol:isopropanol. The plates were then centrifuged at 4 °C for 45 mins and the supernatant carefully decanted with the aid of a multichannel pipette. The oligonucleotide pellet was resuspended in 20 mM NaOAc and subsequently desalted using a HiTrap size exclusion column (5 mL, GE Healthcare) on an Agilent LC system equipped with an autosampler, UV detector, conductivity meter, and fraction collector. Desalted samples were collected in 96 well plates and then analyzed by LC-MS and UV spectrometry to confirm identity and quantify the amount of material, respectively.

Duplexing of single strands was performed on a Tecan liquid handling robot. Sense and antisense single strands were combined in an equimolar ratio to a final concentration of 10 μ M in 1x PBS in 96 well plates, the plate sealed, incubated at 100 °C for 10 minutes, and subsequently allowed to return slowly to room temperature over a period of 2-3 hours. The concentration and identity of each duplex was confirmed and then subsequently utilized for in vitro screening assays.

Example 2. *In vitro* screening methods

Cell culture and 384-well transfections

Hep3b cells (ATCC, Manassas, VA) were grown to near confluence at 37°C in an atmosphere of 5% CO₂ in Eagle's Minimum Essential Medium (Gibco) supplemented with 10% FBS (ATCC) before being released from the plate by trypsinization. Transfection was carried out by adding 14.8 μ L of Opti-MEM plus 0.2 μ L of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) to 5 μ L of each siRNA duplex to an individual well in a 96-well plate. The mixture was then incubated at room temperature for 15 minutes. Eighty μ L of complete growth media without antibiotic containing $\sim 2 \times 10^4$ Hep3B cells were then added to the siRNA mixture. Cells were incubated for 24 hours prior to RNA purification. Single dose experiments were performed at 10 nM and 0.1 nM final duplex concentration and dose response experiments were done using 8x 5-fold serial dilutions over the range of 10 nM to 128 pM.

Total RNA isolation using DYNABEADS mRNA Isolation Kit (Invitrogen™, part #: 610-12)

Cells were lysed in 75 μ L of Lysis/Binding Buffer containing 3 μ L of beads per well and mixed for 10 minutes on an electrostatic shaker. The washing steps were automated on a Biotek EL406, using a magnetic plate support. Beads were washed (in 90 μ L) once in Buffer A, once in Buffer B, and twice in Buffer E, with aspiration steps in between. Following a final aspiration, complete 10 μ L RT mixture was added to each well, as described below.

cDNA synthesis using ABI High capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, Cat #4368813)

A master mix of 1µl 10X Buffer, 0.4µl 25X dNTPs, 1µl Random primers, 0.5µl Reverse Transcriptase, 0.5µl RNase inhibitor and 6.6µl of H₂O per reaction were added per well. Plates were sealed, agitated for 10 minutes on an electrostatic shaker, and then incubated at 37 degrees C for 2 hours. Following this, the plates were agitated at 80 degrees C for 8 minutes.

Real time PCR

Two microlitre (µl) of cDNA were added to a master mix containing 0.5µl of human GAPDH TaqMan Probe (4326317E), 0.5µl human APOC3, 2µl nuclease-free water and 5µl Lightcycler 480 probe master mix (Roche Cat # 04887301001) per well in a 384 well plates (Roche cat # 04887301001). Real time PCR was done in a LightCycler480 Real Time PCR system (Roche).

To calculate relative fold change, data were analyzed using the $\Delta\Delta C_t$ method and normalized to assays performed with cells transfected with 10nM AD-1955, or mock transfected cells. IC₅₀s were calculated using a 4 parameter fit model using XLFit and normalized to cells transfected with AD-1955 or mock-transfected. The sense and antisense sequences of AD-1955 are: sense: cuuAcGcuGAGuAcuucGAdTsdT (SEQ ID NO:22) and antisense UCGAAGuACUcAGCGuAAGdTsdT (SEQ ID NO:23).

The results of the screening of the dsRNA agents listed in Tables 3 and 5 in Hep3B cells are shown in Tables 6 and 7, respectively.

Table 1. Abbreviations of nucleotide monomers used in nucleic acid sequence representation. It will be understood that these monomers, when present in an oligonucleotide, are mutually linked by 5'-3'-phosphodiester bonds.

Abbreviation	Nucleotide(s)
A	Adenosine-3'-phosphate
Ab	beta-L-adenosine-3'-phosphate
Abs	beta-L-adenosine-3'-phosphorothioate
Af	2'-fluoroadenosine-3'-phosphate
Afs	2'-fluoroadenosine-3'-phosphorothioate
As	adenosine-3'-phosphorothioate
C	cytidine-3'-phosphate
Cb	beta-L-cytidine-3'-phosphate
Cbs	beta-L-cytidine-3'-phosphorothioate
Cf	2'-fluorocytidine-3'-phosphate
Cfs	2'-fluorocytidine-3'-phosphorothioate
Cs	cytidine-3'-phosphorothioate
G	guanosine-3'-phosphate
Gb	beta-L-guanosine-3'-phosphate
Gbs	beta-L-guanosine-3'-phosphorothioate
Gf	2'-fluoroguanosine-3'-phosphate
Gfs	2'-fluoroguanosine-3'-phosphorothioate
Gs	guanosine-3'-phosphorothioate
T	5'-methyluridine-3'-phosphate
Tf	2'-fluoro-5-methyluridine-3'-phosphate

Abbreviation	Nucleotide(s)
Tfs	2'-fluoro-5-methyluridine-3'-phosphorothioate
Ts	5-methyluridine-3'-phosphorothioate
U	Uridine-3'-phosphate
Uf	2'-fluorouridine-3'-phosphate
Ufs	2'-fluorouridine-3'-phosphorothioate
Us	uridine-3'-phosphorothioate
N	any nucleotide, modified or unmodified
a	2'-O-methyladenosine-3'-phosphate
as	2'-O-methyladenosine-3'-phosphorothioate
c	2'-O-methylcytidine-3'-phosphate
cs	2'-O-methylcytidine-3'-phosphorothioate
g	2'-O-methylguanosine-3'-phosphate
gs	2'-O-methylguanosine-3'-phosphorothioate
t	2'-O-methyl-5-methyluridine-3'-phosphate
ts	2'-O-methyl-5-methyluridine-3'-phosphorothioate
u	2'-O-methyluridine-3'-phosphate
us	2'-O-methyluridine-3'-phosphorothioate
s	phosphorothioate linkage
L10	N-(cholesterylcarboxamidocaproyl)-4-hydroxyprolinol (Hyp-C6-Chol)
L96	N-[tris(GalNAc-alkyl)-amidodecanoyl]-4-hydroxyprolinol (Hyp-(GalNAc-alkyl)3)
Y34	2-hydroxymethyl-tetrahydrofuran-4-methoxy-3-phosphate (abasic 2'-OMe furanose)
Y44	inverted abasic DNA (2-hydroxymethyl-tetrahydrofuran-5-phosphate)
(Agn)	Adenosine-glycol nucleic acid (GNA)
(Cgn)	Cytidine-glycol nucleic acid (GNA)
(Ggn)	Guanosine-glycol nucleic acid (GNA)
(Tgn)	Thymidine-glycol nucleic acid (GNA) S-Isomer
P	Phosphate
VP	Vinyl-phosphonate
dA	2'-deoxyadenosine-3'-phosphate
dAs	2'-deoxyadenosine-3'-phosphorothioate
dC	2'-deoxycytidine-3'-phosphate
dCs	2'-deoxycytidine-3'-phosphorothioate
dG	2'-deoxyguanosine-3'-phosphate
dGs	2'-deoxyguanosine-3'-phosphorothioate
dT	2'-deoxythymidine-3'-phosphate
dTs	2'-deoxythymidine-3'-phosphorothioate
dU	2'-deoxyuridine
dUs	2'-deoxyuridine-3'-phosphorothioate
(C2p)	cytidine-2'-phosphate
(G2p)	guanosine-2'-phosphate
(U2p)	uridine-2'-phosphate
(A2p)	adenosine-2'-phosphate
(Chd)	2'-O-hexadecyl-cytidine-3'-phosphate
(Ahd)	2'-O-hexadecyl-adenosine-3'-phosphate
(Ghd)	2'-O-hexadecyl-guanosine-3'-phosphate
(Uhd)	2'-O-hexadecyl-uridine-3'-phosphate
i	2'-O-methylionsine-3'-phosphate

Table 2. Unmodified Sense and Antisense Strand Sequences of Apolipoprotein C3 dsRNA Agents

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range	Antisense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range
AD-959917.1	CUUCAGUUCUCCUGAAAAGACUU	24	NM_000040.3_245-265_A21U_s	245-265	AAGUCUUUCAGGGAAACU	25	NM_000040.3_243-265_U1A_as	243-265
AD-959918.1	UUCAGUUCUCCUGAAAAGACUAU	26	NM_000040.3_246-266_C21U_s	246-266	AUAGUCUUUCAGGGAAACUGAAGC	27	NM_000040.3_244-266_G1A_as	244-266
AD-960096.1	CCAAUAAAAGCUGGACAA GAU	28	NM_000040.3_506-526_G21U_s	506-526	AUUCUUGUCCAGCUUUAUUGGA	29	NM_000040.3_504-526_C1A_as	504-526
AD-960064.1	AAAAGGGACAGUAUUCU CAGU	30	NM_000040.3_435-455_s	435-455	ACUGAGAAUACUGUCCCUUUUUA	31	NM_000040.3_433-455_as	433-455
AD-959914.1	AUGGCUUCAGUUCUCCUGAAU	32	NM_000040.3_241-261_G21U_s	241-261	AUUUCAGGGAAACUGAAGC	33	NM_000040.3_239-261_C1A_as	239-261
AD-959941.1	AGCACCGUUAAGGACAA GUU	34	NM_000040.3_270-290_C21U_s	270-290	AAACUUGUCCUUAACGGUGCUCC	35	NM_000040.3_268-290_G1A_as	268-290
AD-960031.1	UAAAAGGGACAGUAUUC UCAU	36	NM_000040.3_434-454_G21U_s	434-454	AUGAGAAUACUGUCCCUUUUAAG	37		432-454
AD-959910.1	ACCGAUGGCUUCAGUUC CCUU	38	NM_000040.3_237-257_G21U_s	237-257	AAGGAAACUGAAGGCCAU	39	NM_000040.3_235-257_C1A_as	235-257
AD-960063.1	UUAAGGACAGUAUUCU CUCU	40	NM_000040.3_433-453_A21U_s	433-453	AGAGAAUACUGUCCCUUUUAAGC	41	NM_000040.3_431-453_U1A_as	431-453
AD-959916.1	GUUCAGUUCUCCUGAAA GACU	42	NM_000040.3_244-264_s	244-264	AGUCUUUCAGGGAAACUGAAGCCA	43	NM_000040.3_242-264_as	242-264
AD-959913.1	GAUGGCUUCAGUUCUCCU GAAU	44	NM_000040.3_240-260_A21U_s	240-260	AUUCAGGGAAACUGAAGC	45	NM_000040.3_238-260_U1A_as	238-260
AD-960066.1	AAGGACAGUAUUCUCA GUGU	46	NM_000040.3_437-457_C21U_s	437-457	ACACUGAGAAUACUGUC	47	NM_000040.3_435-457_G1A_as	435-457
AD-960062.1	CUUAAAAGGGACAGUAU UCUU	48	NM_000040.3_432-452_C21U_s	432-452	AAGAAUACUGUCCCUUUUAAGCA	49	NM_000040.3_430-452_G1A_as	430-452
AD-960093.1	CUCCCAAUAAAGCUGGA CAAU	50	NM_000040.3_503-523_G21U_s	503-523	AUUGUCCAGCUUUAUUGGGAGGC	51	NM_000040.3_501-523_C1A_as	501-523
AD-960061.1	UGCUUAAAAGGGACAGU AUUU	52	NM_000040.3_430-450_C21U_s	430-450	AAAUACUGUCCCUUUUAAGCAAC	53	NM_000040.3_428-450_G1A_as	428-450
AD-960092.1	CCUCCCAAUAAAGCUGG ACAU	54	NM_000040.3_502-522_A21U_s	502-522	AUGUCCAGCUUUAUUGGGAGGC	55	NM_000040.3_500-522_U1A_as	500-522
AD-960030.1	GCUAAAAGGGACAGUA UUCU	56	NM_000040.3_431-451_s	431-451	AGAAUACUGUCCCUUUUAAGCAA	57	NM_000040.1_428-450_as	429-451
AD-80794.6	CUUAAAAGGGACAGUAU	13	NM_000040.1_433-	433-452	UAGAAUACUGUCCCUUU	58	NM_000040.1_433-	433-

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range	Antisense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range
AD-960095.1	UCUA	59	452_C21A_s		UAAAGCA		-452_C21A_s	452
AD-959938.1	CCCAAUAAAGCUGGACAAGAU	61	NM_000040.3_505-525_A21U_s	505-525	AUCUUGUCCAGCUUUUAUUGGGAG	60	NM_000040.3_503-525_U1A_as	503-525
AD-960065.1	CUGGAGCACCCGUUAAAGGACAU	63	NM_000040.3_266-286_A21U_s	266-286	AUGUCCUUAAACGGGUGCUCCAGUA	62	NM_000040.3_264-286_U1A_as	264-286
AD-959907.1	AAAGGGACAGUAUUCUCAGUU	65	NM_000040.3_436-456_G21U_s	436-456	AACUGAGAAUACUGUCCUUUUUA	64	NM_000040.3_434-456_C1A_as	434-456
AD-960094.1	GUGACCGAUGGCUUCAGUUCU	67	NM_000040.3_234-254_C21U_s	234-254	AGAACUGAAGCCAUCCGUACACC	66	NM_000040.3_232-254_G1A_as	232-254
AD-960060.1	UCCCAAUAAAGCUGGACAGU	69	NM_000040.3_504-524_A21U_s	504-524	ACUUGUCCAGCUUUUAUUGGGAGG	68	NM_000040.3_502-524_U1A_as	502-524
AD-959919.1	GGUUGCUUAAAAAGGGACAGUU	71	NM_000040.3_427-447_A21U_s	427-447	AACUGUCCUUUUAAGCAACCUA	70	NM_000040.3_425-447_U1A_as	425-447
AD-959932.1	UCAGUUCUCCUGAAAAGACUACU	73	NM_000040.3_247-267_s	247-267	AGUAGUCUUUCAGGGGAACUGAAG	72	NM_000040.3_245-267_as	245-267
AD-959859.1	AGACUACUGGAGCACCGUUU	75	NM_000040.3_260-280_A21U_s	260-280	AUAACGGUGUCCAGUAUUCUUU	74	NM_000040.3_258-280_U1A_as	258-280
AD-959908.1	CCACCAAGACCGCAAGGAUU	77	NM_000040.3_163-183_G21U_s	163-183	AAUCCUUGCGGUCUUGGUGGCG	76	NM_000040.3_161-183_C1A_as	161-183
AD-959903.1	UGACCGAUGGCUUCAGUUCU	79	NM_000040.3_235-255_C21U_s	235-255	AGAACUGAAGCCAUCCGUCACC	78	NM_000040.3_233-255_G1A_as	233-255
AD-960097.1	CUGGGUGACCGAUGGCUU	81	NM_000040.3_230-250_G21U_s	230-250	AUGAAGCCAUCCGUCACCCAGCC	80	NM_000040.3_228-250_C1A_as	228-250
AD-959912.1	CAUAAAGCUGGACAAAGAGU	83	NM_000040.3_507-527_C21U_s	507-527	ACUUCUUGUCCAGCUUUU	82	NM_000040.3_505-527_G1A_as	505-527
AD-960067.1	CGAUGGCUUCAGUUCU	85	NM_000040.3_239-259_A21U_s	239-259	AUCAGGGAACUGAAGCCUUCGGU	84	NM_000040.3_237-259_U1A_as	237-259
AD-959927.1	AGGACAGUAUUCUCAGU	87	NM_000040.3_438-458_s	438-458	AGCACUGAGAAUACUGUCCUUU	86	NM_000040.3_436-458_as	436-458
AD-960099.1	CUGAAAGACUACUGGAGCACU	89	NM_000040.3_255-275_C21U_s	255-275	AGUGUCCAGUAAGUCUUU	88	NM_000040.3_253-275_G1A_as	253-275
AD-959931.1	AUAAAGCUGGACAAAGAA	91	NM_000040.3_509-529_G21U_s	509-529	AAGUCUUUUGUCCAGCUUUUUU	90	NM_000040.3_507-529_C1A_as	507-529
AD-959879.1	AAGACUACUGGAGCACC	93	NM_000040.3_259-279_A21U_s	259-279	AAACGGUGUCCAGUAGUCUUUC	92	NM_000040.3_257-279_U1A_as	257-279
	UGGCUUCAGUUCUCCUGAAGU		NM_000040.3_242-262_A21U_s	242-262	ACUUCACGGGAACUGAAGCCAU	94		240-262

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range	Antisense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range
AD-960091.1	GCCUCCCAAAUAAAGCUG GACU	95	NM_000040.3_501-521_A21U_s	501-521	AGUCCAGCUUUUAUUGGG AGGCA	96	NM_000040.3_499-521_U1A_as	499-521
AD-959921.1	AGUUCUCCUGAAAGACUA CUGU	97	NM_000040.3_249-269_G21U_s	249-269	ACAGUAGUUCUUCAGGG AACUGA	98	NM_000040.3_247-269_C1A_as	247-269
AD-960102.1	AAGCUGGACAAAGAAAGCU GCUU	99	NM_000040.3_512-532_A21U_s	512-532	AAGCAGCUUCUUGUCCA GCUUUA	100	NM_000040.3_510-532_U1A_as	510-532
AD-80793.6	GCUGGACAAAGAAAGCUGC UAUUA	101	NM_000040.1_515-534_G21A_s	515-534	UAUAGCAGCUUCUUGUC CAGCUU	102	NM_000040.1_513-534_G21A_as	513-534
AD-959925.1	CCUGAAAGACUACUGG AGCU	103	253-273		AGCUCCAGUAGUCUUUC AGGGAA	104	NM_000040.3_251-273_U1A_as	251-273
AD-960098.1	AAUAAAGCUGGACAAAGA AGCU	105	NM_000040.3_508-528_s	508-528	AGCUUCUUGUCCAGCUU UAUUGG	106	NM_000040.3_506-528_as	506-528
AD-959901.1	GGCUGGGUGACCGAUGG CUUU	107	NM_000040.3_228-248_C21U_s	228-248	AAAGCCAUCCGUCACCC AGCCCC	108	NM_000040.3_226-248_G1A_as	226-248
AD-959920.1	CAGUUCUCCUGAAAGACU ACUU	109	NM_000040.3_248-268_G21U_s	248-268	AAGUAGUCUUUCAGGG AACUGAA	110	NM_000040.3_246-268_C1A_as	246-268
AD-959926.1	CCUGAAAGACUACUGGA GCAU	111	NM_000040.3_254-274_C21U_s	254-274	AUGCUCCAGUAGUCUUU CAGGGA	112	NM_000040.3_252-274_G1A_as	252-274
AD-959737.1	CAUCCUAGAGGCAGCU GCUU	113	NM_000040.3_11-31_C21U_s	11-31	AAGCAGCUGCCUCUAGG GAUGAA	114	NM_000040.3_9-31_G1A_as	9-31
AD-960011.1	CUGCCUGAGACCUCAU ACCU	115	NM_000040.3_340-360_C21U_s	340-360	AGUUAUUGAGGUCUCA GGCAGCC	116	NM_000040.3_338-360_G1A_as	338-360
AD-960101.1	AAAGCUGGACAAAGAAGC UGCU	117	NM_000040.3_511-531_s	511-531	AGCAGCUUCUUGUCCAG CUUUUA	118	NM_000040.3_509-531_as	509-531
AD-959923.1	UUCUCCUGAAAGACUACU GGAU	119	NM_000040.3_251-271_G21U_s	251-271	AUCCAGUAGUCUUUCAG GGAACU	120	NM_000040.3_249-271_C1A_as	249-271
AD-960058.1	UAGGUUGCUUAAAAAGGG ACAU	121	NM_000040.3_425-445_G21U_s	425-445	AUGUCCUUUUAAAGCAA CCUACA	122	NM_000040.3_423-445_C1A_as	423-445
AD-959860.1	CACCAAGACCGCAAGG AUGU	123	NM_000040.3_164-184_C21U_s	164-184	ACAUCUUGGGGUCUUU GGUGGC	124	NM_000040.3_162-184_G1A_as	162-184
AD-960059.1	AGGUUGCUUAAAAAGGGA CAGU	125	NM_000040.3_426-446_s	426-446	ACUGUCCUUUUAAAGCA ACCUAC	126	NM_000040.3_424-446_as	424-446
AD-960103.1	AGCUGGACAAAGAGCUG CUUU	127	NM_000040.3_513-533_s	513-533	AUAGCAGCUUCUUGUCC AGCUUU	128	NM_000040.3_511-533_as	511-533
AD-959740.1	CCUAGAGGCAGCUGCU CCAU	129	NM_000040.3_14-34_G21U_s	14-34	AUGGAGCAGCUGCCUCU AGGGAU	130	NM_000040.3_12-34_C1A_as	12-34

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range	Antisense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range
AD-959939.1	UGGAGCACCGUUAAGGA CAAU	131	NM_000040.3_267- 287_G21U_s	267-287	AUUGUCCUUAACGGGUC UCCAGU	132	NM_000040.3_265- 287_C1A_as	265- 287
AD-959865.1	GACCGCCAAAGGAUGCAC UGAU	133	NM_000040.3_170- 190_G21U_s	170-190	AUCAGUGCAUCCUUGGC GGUCUU	134	NM_000040.3_168- 190_C1A_as	168- 190
AD-960100.1	UAAAGCUGGACAAGAAG CUGU	135	NM_000040.3_510- 530_C21U_s	510-530	ACAGCUUCUUGUCCAGC UUUAUU	136	NM_000040.3_508- 530_G1A_as	508- 530
AD-959924.1	UCCUGAAAGACUACUG GAGU	137	NM_000040.3_252- 272_C21U_s	252-272	ACUCCAGUAGUCUUUCA GGGAAC	138	NM_000040.3_250- 272_G1A_as	250- 272
AD-959909.1	GACCGAUGGCUUCAGUU CCCU	139	NM_000040.3_236- 256_s	236-256	AGGGAACUGAAGCCAUC GGUCAC	140	NM_000040.3_234- 256_as	234- 256
AD-959739.1	UCCCUAGAGGCAGCUGC UCCU	141	NM_000040.3_13- 33_A21U_s	13-33	AGGAGCAGCUGCCUCUA GGGAUG	142	NM_000040.3_11- 33_U1A_as	11-33
AD-959911.1	CCGAUGGCUUCAGUUC CUGU	143	NM_000040.3_238- 258_A21U_s	238-258	ACAGGGAACUGAAGCCA UCGGUC	144	NM_000040.3_236- 258_U1A_as	236- 258
AD-960057.1	GUAGGUUGCUUAAAAGG GACU	145	NM_000040.3_424- 444_A21U_s	424-444	AGUCCCUUUUAAGCAAC CUACAG	146	NM_000040.3_422- 444_U1A_as	422- 444
AD-959741.1	CCUAGAGGCAGCUGCUC CAGU	147	NM_000040.3_15- 35_G21U_s	15-35	ACUGGAGCAGCUGCCUC UAGGGA	148	NM_000040.3_13- 35_C1A_as	13-35
AD-960056.1	UGUAGGUUGCUUAAAAG GGAU	149	NM_000040.3_423- 443_C21U_s	423-443	AUCCCUUUUAAGCAACC UACAGG	150	NM_000040.3_421- 443_G1A_as	421- 443
AD-959930.1	AAAGACUACUGGAGCAC CGUU	151	NM_000040.3_258- 278_s	258-278	AACGGUGCUCCAGUAGU CUUUA	152	NM_000040.3_256- 278_as	256- 278
AD-959746.1	AGGCAGCUGCUCCAGGA ACAU	153	NM_000040.3_20- 40_G21U_s	20-40	AUGUCCUGGAGCAGCU GCCUCU	154	NM_000040.3_18- 40_C1A_as	18-40
AD-959748.1	GCAGCUGCUCCAGGAAC AGAU	155	NM_000040.3_22- 42_G21U_s	22-42	AUCGUUCCUGGAGCAG CUGCCU	156	NM_000040.3_20- 42_C1A_as	20-42
AD-959857.1	CGCCACCAAGACCGCCAA GGU	157	NM_000040.3_161- 181_A21U_s	161-181	ACCUUGGCGGUCUUGGU GGCGUG	158	NM_000040.3_159- 181_U1A_as	159- 181
AD-959935.1	CUACUGGAGCACCCGUUA AGGU	159	NM_000040.3_263- 283_A21U_s	263-283	ACCUUACGGUGUCUCCA GUAGUC	160	NM_000040.3_261- 283_U1A_as	261- 283
AD-960008.1	UGGCUCCUGAGACCUC AAUU	161	NM_000040.3_337- 357_A21U_s	337-357	AAUUGAGGUCUCAGGCA GCCACG	162	NM_000040.3_335- 357_U1A_as	335- 357
AD-959915.1	GGCUUCAGUCCUUGAA AGAU	163	NM_000040.3_243- 263_C21U_s	243-263	AUCUUACAGGGAACUGA AGCCAU	164	NM_000040.3_241- 263_G1A_as	241- 263
AD-959738.1	AUCCCUAGAGGCAGCUG CUCU	165	NM_000040.3_12- 32_C21U_s	12-32	AGAGCAGCUGCCUCUAG GGAUGA	166	NM_000040.3_10- 32_G1A_as	08-32
AD-	UGAAAGACUACUGGAGC	167	NM_000040.3_256-	256-276	AGGUGCUCCAGUAGUCU	168	NM_000040.3_254-	254-

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range	Antisense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range
959928.1	ACCU		276_G21U_s		UUCAGG		276_C1A_as	276
AD-959863.1	AAGACCGCCAAAGGAUGC ACUU	169	NM_000040.3_168-188_G21U_s	168-188	AAGUGCAUCCUUGGCGG UCUUGG	170	NM_000040.3_166-188_C1A_as	166-188
AD-960010.1	GCUGCCUGAGACCUCAA UACU	171	NM_000040.3_339-359_C21U_s	339-359	AGUAUUGAGGUCUCAG GCAGCCA	172	NM_000040.3_337-359_G1A_as	337-359
AD-960090.1	GGCCUCCCAAUAAAGCU GGAU	173	NM_000040.3_500-520_C21U_s	500-520	AUCCAGCUUUAUUGGGA GGCCAG	174	NM_000040.3_498-520_G1A_as	498-520
AD-959732.1	CAGUUCAUCCCUAGAGG CAGU	175	NM_000040.3_6-26_C21U_s	6-26	ACUGCCUCUAGGGGAUGA ACUGAG	176	NM_000040.3_4-26_G1A_as	4-26
AD-960009.1	GGCUGCCUGAGACCUCA AUUU	177	NM_000040.3_338-358_C21U_s	338-358	AUAUUGAGGUCUCAGGC AGCCAC	178	NM_000040.3_336-358_G1A_as	336-358
AD-959929.1	GAAAGACUACUGGAGCA CCGU	179	NM_000040.3_257-277_s	257-277	ACGGUGCUCCAGUAGUC UUUCAG	180	NM_000040.3_255-277_as	255-277
AD-959745.1	GAGGCAGCUGCUCCAGG AACU	181	NM_000040.3_19-39_A21U_s	19-39	AGUUCUGGAGCAGCUG CCUCUA	182	NM_000040.3_17-39_U1A_as	17-39
AD-960007.1	GUGGCTGCCUGAGACCU CAAU	183	NM_000040.3_336-356_s	336-356	AUUGAGGUCUCAGGCAG CCACGG	184	NM_000040.3_334-356_as	334-356
AD-959902.1	GCUGGGUGACCGAUGGC UUCU	185	NM_000040.3_229-249_A21U_s	229-249	AGAAAGCAUCGGGUCACC CAGCCC	186	NM_000040.3_227-249_U1A_as	227-249
AD-959940.1	GGAGCACCGUUAAGGAC AAGU	187	NM_000040.3_268-288_s	268-288	ACUUGUCCUUAACGGUG CUCCAG	188	NM_000040.3_266-288_as	266-288
AD-960055.1	CUGUAGGUUGCUUAAAA GGGU	189	NM_000040.3_422-442_A21U_s	422-442	ACCCUUUU/AAGCAACCU ACAGGG	190	NM_000040.3_420-442_U1A_as	420-442
AD-959922.1	GUUCCCUAGAAAGACUAC UGGU	191	NM_000040.3_250-270_A21U_s	250-270	ACCAGUAGUCUUUUCAGG GAACUG	192	NM_000040.3_248-270_U1A_as	248-270
AD-959900.1	GGGUGGGUGACCGAUG GCUU	193	NM_000040.3_227-247_s	227-247	AAGCCAUCGGUACACCCA GCCCCU	194	NM_000040.3_225-247_as	225-247
AD-959858.1	GCCACCAAGACCGCCAA GGAU	195	NM_000040.3_162-182_s	162-182	AUCCUUGGGGUCUUGG UGGCGU	196	NM_000040.3_160-182_as	160-182
AD-959744.1	AGAGGCAGCUGCUCCAG GAAU	197	NM_000040.3_18-38_C21U_s	18-38	AUUCUUGGAGCAGCUCG CUCUAG	198	NM_000040.3_16-38_G1A_as	16-38
AD-959736.1	UCAUCCCUAGAGGCAGC UGCU	199	NM_000040.3_10-30_s	10-30	AGCAGCUGCCUUCUAGGG AUGAAC	200	NM_000040.3_8-30_as	8-30
AD-959735.1	UUCAUCCCUAGAGGCAG CUGU	201	NM_000040.3_9-29_C21U_s	9-29	ACAGCUGCCUUCUAGGGA UGAACU	202	NM_000040.3_7-29_G1A_as	29-Jul
AD-960039.1	CGAGCUCCUUGGGUCCU GCAU	203	NM_000040.3_386-406_A21U_s	386-406	AUGCAGGACCCAAAGGAG CUCGCA	204	NM_000040.3_384-406_U1A_as	384-406

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range	Antisense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range
AD-959747.1	GGCAGCUGCUCACAGGAA CAGU	205	NM_000040.3_21-41_A21U_s	21-41	ACUGUCCUGGAGCAGC UGCCUC	206	NM_000040.3_19-41_U1A_as	19-41
AD-959862.1	CCAAGACCGCCAAAGGAU GCAU	207	NM_000040.3_166-186_C21U_s	166-186	AUGCAUCCUUGGCGGUC UUGGUG	208	NM_000040.3_164-186_G1A_as	164-186
AD-959933.1	GACUACUGGAGCACCUGU UAAU	209	NM_000040.3_261-281_G21U_s	261-281	AUUAACGGGUGCUCCAGU AGUCUU	210	NM_000040.3_259-281_C1A_as	259-281
AD-959733.1	AGUUCAUCCCUAGAGGC AGCU	211	NM_000040.3_7-27_s	7-27	AGCUGCCUCUAGGGAUG AACUGA	212	NM_000040.3_5-27_as	5-28
AD-959937.1	ACUGGAGCACCGUUAAG GACU	213	NM_000040.3_265-285_A21U_s	265-285	AGUCCUUAACGGGUGCUC CAGUAG	214	NM_000040.3_263-285_U1A_as	263-285
AD-959904.1	UGGGUGACCGAUGGCUU CAGU	215	NM_000040.3_231-251_s	231-251	ACUGAAGCCAUCCGGUCA CCCAGC	216	NM_000040.3_229-251_as	229-251
AD-959797.1	CCGAGCUUCAGAGGCCG AGGU	217	NM_000040.3_101-121_A21U_s	101-121	ACCUCGGCCUCUGAAGC UCGGGC	218	NM_000040.3_99-121_U1A_as	99-121
AD-959861.1	ACCAAGACCGCCAAAGGA UGCU	219	NM_000040.3_165-185_A21U_s	165-185	AGCAUCCUUGGCGGUCU UGGUGG	220	NM_000040.3_163-185_U1A_as	163-185
AD-959743.1	UAGAGGCAGCUGCUCCA GGAU	221	NM_000040.3_17-37_A21U_s	17-37	AUCCUGGAGCAGCUGCC UCUAGG	222	NM_000040.3_15-37_U1A_as	15-37
AD-959905.1	GGGUGACCGAUGGCUUC AGUU	223	NM_000040.3_232-252_s	232-252	AACUGAAGCCAUCCGGUC ACCCAG	224	NM_000040.3_230-252_as	230-252
AD-959734.1	GUUCAUCCCUAGAGGCA GCUU	225	NM_000040.3_8-28_G21U_s	8-28	AAGCUGCCUCUAGGGAU GAACUG	226	NM_000040.3_6-28_C1A_as	6-28
AD-959934.1	ACUACUGGAGCACCCGUU AAGU	227	NM_000040.3_262-282_G21U_s	262-282	ACUUAACGGUGCUCCAG UAGUCU	228	NM_000040.3_260-282_C1A_as	260-282
AD-959749.1	CAGCUGCUCCAGGAACA GAGU	229	NM_000040.3_23-43_G21U_s	23-43	ACUCUGUCCUGGAGCA GCUGCC	230	NM_000040.3_21-43_C1A_as	21-43
AD-959798.1	CGAGCUUCAGAGGCCGA GGAU	231	NM_000040.3_102-122_s	102-122	AUCCUGGCCUCUGAAG CUCGGG	232	NM_000040.3_100-122_as	100-122
AD-959742.1	CUAGAGGCAGCUGCUCC AGGU	233	NM_000040.3_16-36_A21U_s	16-36	ACCUGGAGCAGCUGCCU CUAGGG	234	NM_000040.3_14-36_U1A_as	14-36
AD-959897.1	GAGUCCAGGUGGCCCA GCAU	235	NM_000040.3_201-221_G21U_s	201-221	AUGCUGGCCACCUGGG ACUCCU	236	NM_000040.3_199-221_C1A_as	199-221
AD-959864.1	AGACCGCCAAAGGAUGCA CUGU	237	NM_000040.3_169-189_A21U_s	169-189	ACAGUGCAUCCUUGGCG GUCUUG	238	NM_000040.3_167-189_U1A_as	167-189
AD-959899.1	CCAGGUGGCCAGCAGG CCAU	239	NM_000040.3_206-226_G21U_s	206-226	AUGGCCUGCUGGGCCAC CUGGGA	240	NM_000040.3_204-226_C1A_as	204-226
AD-	ACGCCACCAAGACCGCCA	241	NM_000040.3_160-180_s	160-180	ACUUGGGGUCUUGGUG	242	NM_000040.3_158-180_s	158-

Duplex ID	Sense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range	Antisense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range
959856.1	AGU		180_G21U_s		GCGUGC		180_C1A_as	180
AD-959906.1	GGUACCGAUGGCUUCA GUUU	243	NM_000040.3_233- 253_C21U_s	233-253	AAACUGAAGCCAUCCGU CACCCA	244	NM_000040.3_231- 253_G1A_as	231- 253
AD-959936.1	UACUGGAGCACCGUAAA GGAU	245	NM_000040.3_264- 284_C21U_s	264-284	AUCCUUAACGGUGCUCC AGUAGU	246	NM_000040.3_262- 284_G1A_as	262- 284
AD-959986.1	GGAGUCCACAGGUGGCC AGCU	247	NM_000040.3_200- 220_A21U_s	200-220	AGCUGGCCACCUUGGA CUCCUG	248	NM_000040.3_198- 220_U1A_as	198- 220
AD-959893.1	GCAGGAGUCCACAGGUG CCCU	249	NM_000040.3_197- 217_A21U_s	197-217	AGGGCCACCUUGGACUC CUGCAC	250	NM_000040.3_195- 217_U1A_as	195- 217
AD-959892.1	UGCAGGAGUCCACAGGUG GCCU	251	NM_000040.3_196- 216_C21U_s	196-216	AGGCCACCUUGGACUCC UGCACG	252	NM_000040.3_194- 216_G1A_as	194- 216
AD-959894.1	CAGGAGUCCACAGGUGGC CCAU	253	NM_000040.3_198- 218_G21U_s	198-218	AUGGCCACCUUGGACU CCUGCA	254	NM_000040.3_196- 218_C1A_as	196- 218
AD-959750.1	AGCUGCUCCAGGAACAG AGGU	255	NM_000040.3_24- 44_s	24-44	ACCUCGUUCCUGGAGC AGCUGC	256	NM_000040.3_22- 44_as	22-44
AD-959898.1	AGUCCACAGGUGGCCACAG CAGU	257	NM_000040.3_202- 222_G21U_s	202-222	ACUCUGGGCCACCUUG GACUCC	258	NM_000040.3_200- 222_C1A_as	200- 222
AD-959895.1	AGGAGUCCACAGGUGGCC CAGU	259	NM_000040.3_199- 219_C21U_s	199-219	ACUGGCCACCUUGGAC UCCUGC	260	NM_000040.3_197- 219_G1A_as	197- 219

Table 3. Modified Sense and Antisense Strand Sequences of Apolipoprotein C3 dsRNA Agents

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-959917.1	csusucagUfuCfCfCfugaagaauL96	341	asAfsagcUfuUfCfaggGfAfaCfugaagcsc	342	GGCUUCAGUUCUCCUGAAAGACUA	343
AD-959918.1	ususcaguUfCfCfUfgaagacuaL96	344	asUfsagnCfuUfUfcaggGfAfaCfugaagcsc	345	GCUUCAGUUCUCCUGAAAGACUAC	346
AD-960096.1	cscsaauaAfaGfCfUfggacaagaauL96	347	asUfsucuUfgUfCfcagcUfuUfaunggsgsa	348	UCCCAAUAAAGCUGGACAAAGAAG	349
AD-960064.1	asasaaggGfaCfAfGfuauucacauL96	350	asCfsugaGfaAfUfaucgUfcCfuuuuasasa	351	UUAAAAAGGGACAGUAUUCUCAGU	352
AD-959914.1	asusggcuUfCfAfGfUfucccugaauL96	353	asUfsuucAfgGfGfaacuGfaAfGCCauscsg	354	CGAUGGCUUCAGUUCUCCUGAAAG	355
AD-959941.1	asgscaccGfuUfAfAfgacaaguuL96	356	asAfsacuUfgUfCfcmaAfcGfgugcuscsc	357	GGAGCACCCGUUAAAGGACAAAGUUC	358
AD-960031.1	usasaagGfCfAfCfAfgauucacauL96	359	asUfsagGfAfaUfAfcuGfCfcfuuuuasag	360	CUUAAAAAGGGACAGUAUUCUCAG	361
AD-959910.1	ascscgauGfGfCfUfUfCfaguuccuuL96	362	asAfsaggGfAfaCfUfgaagCfCfAfgugcscsa	363	UGACCGAUGGCUUCAGUUCUCCUG	364

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-960063.1	ususaGfGfAfCfaguanuucuuL96	365	asGfsagaAfuAfCfugucCfcUfuuaagsgc	366	GCUUAAAAGGACAGUAUUCUCA	367
AD-959916.1	gscsuucaGfuUfCfCfcuagaagacuL96	368	asGfsuucUfuCfAfgggaAfcUfgaagcscsa	369	UGGCUUCAGUUCUCCUGAAAAGACU	370
AD-959913.1	gsasuggcUfuCfAfGfhucccugauL96	371	asUfsucaGfGfAfacugAfaGfcaucsgsg	372	CCGAUGGCUUCAGUUCUCCUGAAA	373
AD-960066.1	asasgggaCfaGfUfAfuucucagugL96	374	asCfsacuGfGfAfauacUfgUfcccususu	375	AAAAGGACAGUAUUCUCAGUGC	376
AD-960062.1	csusuaaaAfgGfAfcauauucuuL96	377	asAfsaaUfaCfUfguccCfuUfuuaagscsa	378	UGCUUAAAAGGACAGUAUUCUC	379
AD-960093.1	csuscccaAfuAfAfAfgcugacaauL96	380	asUfsuguCfcAfGfcuuuAfuUfgggagsgsc	381	GCCUCCCAAUAAAAGCUGGACAAAG	382
AD-960061.1	usgscuuAfaAfGfGfagacuaauuL96	383	asAfsauaCfuGfUfcccUfuUfaagcasc	384	GUUGCUUAAAAGGACAGUAUUC	385
AD-960092.1	cscsucccAfaUfAfAfagcuggacauL96	386	asUfsgucCfaGfCfuuuaUfuGfggagsgsc	387	GGCUCCCAAUAAAAGCUGGACAA	388
AD-960030.1	gscsuuaaAfaGfGfGfagacuaauL96	389	asGfsauAfcUfGfuccUfuUfuuaagcsasa	390	UUGCUUAAAAGGACAGUAUUCU	391
AD-80794.6	csusuaaaAfgGfAfcauauucuaL96	17	usAfsaaUfaCfUfguccCfuUfuuaagscsa	392	CUUAAAAGGACAGUAUUCUA	393
AD-960095.1	cscscuuAfaAfGfCfuggacaaauL96	394	asUfscuuGfuCfCfaguuUfuAfuugggsasg	395	CUCCCAAUAAAAGCUGGACAAAGAA	396
AD-959938.1	csusgggagCfaCfCfGfuaaagacauL96	397	asUfsgucCfuUfAfacggUfgCfuccagsusa	398	UACUGGAGCACCGUUAAGGACAA	399
AD-960065.1	asasaggAfcAfGfUfaucucaguuL96	400	asAfsaugAfgAfAfuacuGfuCfcccuisusa	401	UAAAAGGACAGUAUUCUCAGUG	402
AD-959907.1	gsusgaccGfaUfGfGfucucaguuL96	403	asGfsaacUfgAfAfgecaUfcGfgeucacsc	404	GGGUGACCGAUGGCUUCAGUUC	405
AD-960094.1	uscsccaaUfaAfAfGfuggacaaauL96	406	asCfsuugUfcCfAfgeuuUfaUfugggasgsg	407	CCUCCCAAUAAAAGCUGGACAAAGA	408
AD-960060.1	gsgsuugcUfuAfAfAfagggacaguuL96	409	asAfsaugUfcCfCfuuuuAfaGfcaaccsusa	410	UAGGUUGCUUAAAAGGACAGUA	411
AD-959919.1	uscaguuCfcCfUfGfaaagacuL96	412	asGfsuagUfcUfUfucagGfgAfacuigasasg	413	CUUCAGUUCUCCUGAAAAGACUACU	414
AD-959932.1	asgsacuaCfuGfGfAfgcaccguauL96	415	asUfsaacGfGfUfGfuccAfGfagucuuusu	416	AAAAGACUACUGGAGCACCGUUA	417
AD-959859.1	cscsaccaAfgAfCfCfcccaggaauL96	418	asAfsuccUfuGfGfaggUfuUfggugsgsg	419	CGCCACCAAGACCCGCCAAGGAUG	420
AD-959908.1	usgsaccgAfuGfGfCfuucaguuL96	421	asGfsagaaCfuGfAfagccAfuCfagucacsc	422	GGUGACCGAUGGCUUCAGUUC	423
AD-959903.1	csusggguGfaCfCfGfagggcucauL96	424	asUfsgaaGfcCfAfucggUfcAfcccagscsc	425	GGCUGGGUGACCGAUGGCUUCAG	426
AD-960097.1	csasuaaaAfgCfUfGfagacaagaauL96	427	asCfsuucUfuGfUfcccagCfuUfuuaugsgsg	428	CCCAUAAAAGCUGGACAAAGAGC	429
AD-959912.1	csgsauggCfuUfCfAfguuuccuauL96	430	asUfscagGfgAfAfcuagAfgCfcaucsgsgsu	431	ACCGAUGGCUUCAGUUCUCCUGAA	432
AD-960067.1	asgsggacAfgUfAfUfucucaguuL96	433	asGfscacUfgAfGfaauaCfuGfuccuuusu	434	AAAAGGACAGUAUUCUCAGUGCU	435
AD-959927.1	csusgaaaGfaCfUfAfcuggagacauL96	436	asGfsugeUfcCfAfaguagUfcUfuucagsgsg	437	CCCUGAAAAGACUACUGGAGCAC	438
AD-960099.1	asusaaagCfuGfGfAfcagaagcuuL96	439	asAfsgeuUfcUfUfguccAfgCfuuuausug	440	CAAUAAAAGCUGGACAAAGAAAGCUG	441

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-959931.1	asasgacuAfcUfGfGfagcagcguuuL96	442	asAfsagGfuGfCfucaGfuAfgucuuusc	443	GAAGACUACUGGAGCACCCGUUA	444
AD-959879.1	usgsgeuuCfaGfUfUfccccgaaaguL96	445	asCfsuuuCfaGfGfgaacUfgAfgaccasusc	446	GAUGGCUUACAGUUCUCCUGAAAAGA	447
AD-960091.1	gscsucceCfaAfuAfaagcugacuL96	448	asGfsuccAfcCfUfuuuUfgGfagccscsa	449	UGGCCUCCCAAUAAAAGCUGGACA	450
AD-959921.1	asgsnuccCfuGfAfAfaagacuacuL96	451	asCfsaguAfgUfCfuuuAfgGfgaacugsa	452	UCAGUUCUCCUGAAAAGACUACUGG	453
AD-960102.1	asasgcugGfaCfAfAfgaagcugcuuL96	454	asAfsgeaGfUfUfuuuUfgCfagcuususa	455	UAAAGCUGGACAAAGAAAGCUGCUA	456
AD-80793.6	gscsuaggGfaAfuAfaagcugcuuL96	457	usAfsuagCfaGfCfuuuUfgUfagccsusu	458	GCUGGACAAAGAAAGCUGCUAAUA	459
AD-959925.1	cscscugaAfaGfAfCfuacugagcuL96	460	asGfscucCfaGfUfagucUfuUfagccgsasa	461	UUUCCUGAAAAGACUACUGGAGCA	462
AD-960098.1	asasuaaaGfcUfGfGfagcagagcuL96	463	asGfscuuCfuUfGfuceaGfcUfuuaauusg	464	CCAUAUAAAGCUGGACAAAGAAAGCU	465
AD-959901.1	gsgscugGfuGfAfCfagcugcguuL96	466	asAfsagcCfaUfCfagucAfcCfagccscsc	467	GGGGCUGGGUGACCCGAUGGCUUC	468
AD-959920.1	csasgmuuCfcUfGfAfaagacuacuL96	469	asAfsguaGfuCfUfuucaGfgGfagcuggsasa	470	UUCAGUUCUCCUGAAAAGACUACUG	471
AD-959926.1	cscsugaaAfgAfCfUfagcugagcuL96	472	asUfsgcuCfcAfGfuaguCfuUfucaggsasa	473	UCCUGAAAAGACUACUGGAGCAC	474
AD-959737.1	csasuccUfaGfAfGfagcugcguuL96	475	asAfsgeaGfUfGfuccUfGfagcuggsasa	476	UUCUCCCUAGAGGCAGCUGCUUC	477
AD-960011.1	csusgcuGfaGfAfCfoucauacuL96	478	asGfsguaUfuGfAfagucUfcAfggcagcsc	479	GGCUGCCUGAGACCUCUCAAUACCC	480
AD-960101.1	asasagcuGfgAfCfAfaagcugcuL96	481	asGfscagCfuUfCfuuguCfcAfgeuuusasu	482	AUAAGCUGGACAAAGAAAGCUGCU	483
AD-959923.1	ususcccuGfaAfAfGfacuacuggauL96	484	asUfscgaGfuAfGfucuuUfcAfgggaacsu	485	AGUUCUCCUGAAAAGACUACUGGAG	486
AD-960058.1	usasgguuGfcUfUfAfaagggacuL96	487	asUfsgucCfcUfUfuuaaGfcAfaccuascsa	488	UGUAGGUUGCUUAAAAGGGACAG	489
AD-959860.1	csasccaaGfaCfCfGfccaaggaugL96	490	asCfsaucCfuUfGfagcggUfcUfugcuggsa	491	GCCACCAAGACCCGCCAAGGAUGC	492
AD-960059.1	asgsuugCfuUfAfAfaagggacaguL96	493	asCfsuguCfcUfUfuuaaAfgCfaaccusasc	494	GUAGGUUGCUUAAAAGGGACAGU	495
AD-960103.1	asgsccugAfcAfAfGfagcugcguuL96	496	asUfsagcAfgCfUfucuuGfuCfagcucusu	497	AAAAGCUGGACAAAGAAAGCUGCUAU	498
AD-959740.1	cscscuagAfgGfCfAfgcugcuccauL96	499	asUfsggaGfcAfGfcugcCfuCfuagggsasu	500	AUCCCUAGAGGCAGCUGCUCCAG	501
AD-959939.1	usgsagcAfcCfGfUfuaaggaacuL96	502	asUfsguGfcUfUfagcGfuGfuccacgsu	503	ACUGGAGCACCCGUUUAAGGACAAG	504
AD-959865.1	gsasccgcCfaAfGfGfagcagcaguL96	505	asUfscagUfgCfAfucuuUfgGfagcucusu	506	AAGACCGCCAAAGGAUGCAGCUGAG	507
AD-960100.1	usasaaagUfgGfAfCfaaggaagcuL96	508	asCfsagcUfuCfUfugucCfaGfcuuuasusu	509	AAUAAAAGCUGGACAAAGAAAGCUGC	510
AD-959924.1	uscscuagAfaAfGfAfouacuggaugL96	511	asCfsuccAfgUfAfngcuUfuCfagggasasc	512	GUUCCCUGAAAAGACUACUGGAGC	513
AD-959909.1	gsasccgaUfgGfCfUfucaggucccuL96	514	asGfsggaAfcUfGfagcCfaUfagcucasc	515	GUGACCGAUGGCUUACAGUUCUCCU	516
AD-959739.1	uscscuuaGfaGfCfagcugcuccuL96	517	asGfsgagCfaGfCfagccUfcUfagggasug	518	CAUCCCUAGAGGCAGCUGCUCCA	519

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-959911.1	cscsgaugGfcUfUfCfaguuuccuL96	520	asCfsagGfAfcUfuaGfCfCfauccgsc	521	GACCGAUGGCUUCAGUCCCCUGA	522
AD-960057.1	gsusagguUfgCfUfUfaaaagggaL96	523	asGfsuccCfuUfUfuaagCfAfcuacsa	524	CUGUAGGUUUGCUUAAAAAGGGACA	525
AD-959741.1	cscsuagaGfcCfAfGfcuucccauL96	526	asCfsuagAfGfcUfCfagcCfUfucaggsgsa	527	UCCCUAGAGGCAGCUGCUCCAGG	528
AD-960056.1	usguaggUfuGfCfUfuaaaaggauL96	529	asUfseccUfuUfUfuaagCfAfcuacsa	530	CCUGUAGGUUGCUUAAAAAGGGAC	531
AD-959930.1	asasagacUfaCfUfGfagaccgcuL96	532	asAfsccgUfgCfUfcccagUfaGfucuuusca	533	UGAAAAGACUACUGGAGCACCGUU	534
AD-959746.1	asgsagcCfuGfCfUfocaggacauL96	535	asUfsguuCfcUfGfagcAfgCfugccscsu	536	AGAGGCAGCUGCUCCAGGAACACAG	537
AD-959748.1	gscsagcuGfcUfCfCfaggaacauL96	538	asUfscuUfuCfCfuggaGfcAfgcugscsu	539	AGGCAGCUGCUCCAGGAACACAGAG	540
AD-959857.1	csgscacCfaAfGfAfccgcaagauL96	541	asCfscuuGfgCfGfgcuUfgGfugcgscusg	542	CACGCCACCAAGACCAGGCAAGGA	543
AD-959935.1	csusacugGfaGfCfAfccgnaagauL96	544	asCfscuuAfaCfGfgucUfcCfagnagsc	545	GACUACUGGAGCACCGUUAAAGGA	546
AD-960008.1	usgsuagCfcUfGfAfagaccuauL96	547	asAfsuugAfGfUfcaGfCfagccscsg	548	CGUUGCUGCCUGAGACCUCUAAUA	549
AD-959915.1	gsgscuucAfgUfUfCfcuagaagauL96	550	asUfscuuUfcAfgGfgaaCfuGfaagccsa	551	AUGGCUUCAGUCCCCUGAAAGAC	552
AD-959738.1	asusccuUfgAfGfGfcagcugcuL96	553	asGfsagCfUfUfGfcUfUfgggausgsa	554	UCAUCCCUAGAGGCAGCUGCUCC	555
AD-959928.1	usgsaaagAfcUfAfcfugagaccuL96	556	asGfsgugCfuCfCfagnuaGfuCfuucagsg	557	CCUGAAAGACUACUGGAGCACCG	558
AD-959863.1	asasgaccGfcCfAfAfggaugcauL96	559	asAfsugCfaUfCfcuugGfcGfgcuusgs	560	CCAAGACCCGCCAAGGAUGCACUG	561
AD-960010.1	gscsuagUfgAfGfAfccuacauL96	562	asGfsuanUfgAfGfgcuCfaGfagccsa	563	UGGCUGCCUGAGACCUCUAAUACC	564
AD-960090.1	gsgscuucCfcAfAfUfaaagcugauL96	565	asUfscacGfcUfUfuaUfgGfagccscasg	566	CUGGCCUCCCAAUAAAGCUGGAC	567
AD-959732.1	csasguucAfuCfCfCfuagagcaguL96	568	asCfsugCfuCfUfagggAfuGfaacugsa	569	CUCAGUUCAUCCCUAGAGGCAGC	570
AD-960009.1	gsgscugCfuGfAfGfaccuacauL96	571	asUfsaUfGfaGfGfuencAfgGfagccsc	572	GUGGCUGCCUGAGACCUCUAAUAC	573
AD-959929.1	gsasaagaCfuAfCfUfaggagaccuL96	574	asCfsgguGfcUfCfagauAfgUfuuuacsag	575	CUGAAAGACUACUGGAGCACCGU	576
AD-959745.1	gsasggcaGfcUfGfCfuaccagauL96	577	asGfsmucCfuGfGfagcaGfcUfcccusa	578	UAGAGGCAGCUGCUCCAGGAACA	579
AD-960007.1	gsusggcuGfcCfUfGfagaccuauL96	580	asUfsguGfgUfCfuaagGfcAfgcaacs	581	CCGUGGCUGCCUGAGACCUCUAAU	582
AD-959902.1	gscsuaggUfgAfCfCfagggcuuL96	583	asGfsaagCfcAfUfegguCfaCfagccsc	584	GGGCUGGGUGACCUGGCUUUA	585
AD-959940.1	gsgsagcaCfcGfUfUfaaggaagauL96	586	asCfsuugUfcCfUfuaacGfgUfuccscasg	587	CUGGAGCACCGUUAAAGGACAAAGU	588
AD-960055.1	csusguagGfuUfGfCfuuaaaaggauL96	589	asCfscuuUfuUfUfagcaAfcCfuacags	590	CCCUGUAGGUUGCUUAAAAAGGGA	591
AD-959922.1	gsusuccUfgAfAfAfgaauuL96	592	asCfscagUfaGfUfuuuUfCfaggaacs	593	CAGUCCCCUGAAAGACUACUGGA	594
AD-959900.1	gsgsgcuGfgUfGfAfccgaugcuuL96	595	asAfsgccAfuCfGfagcaCfcCfagccscsu	596	AGGGGCUGGGUGACCUGGCUU	597

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-959858.1	gscsaccAfaGfAfCfcgccaaggaauL96	598	asUfscuuUfgGfCfGgucUfuGfGgugcsgsu	599	ACGCCACCAAGACCGCCAAAGGAU	600
AD-959744.1	asgsagacAfgCfUfGfucuccaggaauL96	601	asUfsuccUfgGfAfGcagCfuGfGcucuasg	602	CUAGAGGACAGCUGCUCACAGGAAC	603
AD-959736.1	uscsauccCfuAfGfAfGcagcagcuL96	604	asGfscagCfuGfCfcuuAfGfGfaggaasac	605	GUUCAUCCCUAGAGGCAGCUGCU	606
AD-959735.1	ususeaucCfcUfAfGfagcagcuL96	607	asCfsagcUfgCfCfucaAfGfGfaggaascsu	608	AGUUCAUCCCUAGAGGCAGCUGC	609
AD-960039.1	csGsaagcCfcUfUfGfGgucucgcauL96	610	asUfsgcaGfGfAfCfcaaaGfGfGcucgcsa	611	UGCGAGCUCUUGGGUCCUGCAA	612
AD-959747.1	gsgscagcUfgCfUfCfcaggaacagcuL96	613	asCfsuagUfcCfUfGgagCfaGfGcucscsc	614	GAGGCAGCUGCUCUCCAGGAACAGA	615
AD-959862.1	cscaagaCfcGfCfCfaaggaucagcuL96	616	asUfsgcaUfcCfUfugcGfGfUfcuugcsug	617	CACCAAGACCGCCAAAGGAUGCAC	618
AD-959933.1	gsascuacUfgGfAfGfcaacgguuauL96	619	asUfsuaaCfGfGfUfGcucCfaGfuaucsuu	620	AAGACUACUGGAGCACCCGUUAAG	621
AD-959733.1	asgsuucUfcCfCfUfagagcgagcuL96	622	asGfscugCfcUfCfuaggGfaUfGaaucgsa	623	UCAGUUCAUCCCUAGAGGCAGCU	624
AD-959937.1	ascsuaggGfcAfCfCfGuaaaggaucL96	625	asGfscucUfuAfAfCfGguGfCfUfcagucag	626	CUACUGGAGCACCCGUUAAGGACA	627
AD-959904.1	usgsagugAfcCfGfAfuggcucagcuL96	628	asCfsuagAfcCfCfagcGfUfCfacecagsc	629	GCUGGGUGACCCGAUGGCUUCAGU	630
AD-959797.1	csGsgagcUfuCfAfGfagggccagcuL96	631	asCfscucGfGfCfCfucgAfaGfGcucgsgsc	632	GCCGAGCUUCAGAGGCCGAGGA	633
AD-959861.1	ascscagAfcCfGfCfcaaggaucguL96	634	asGfscuacCfcUfUfGgagGfuCfuugcsgsg	635	CCACCAAGACCGCCAAAGGAUGCA	636
AD-959743.1	usasgagcCfaGfCfUfGcuccaggaauL96	637	asUfscuuGfGfAfGfGcagUfgCfCucuasgsg	638	CCUAGAGGCAGCUGCUCACAGGA	639
AD-959905.1	gsGsgugaCfcGfAfUfGgcuucagcuL96	640	asAfsuagAfaGfCfcaucGfGfUfcacccasag	641	CUGGGUGACCGAUUGGCUUCAGUU	642
AD-959734.1	gsuucuuCfcCfUfAfGfagcgagcuL96	643	asAfsuagCfcCfUfCfagGfGfAfGuaacscsg	644	CAGUUCAUCCCUAGAGGCAGCUG	645
AD-959934.1	ascsuacuGfGfAfGfCfacegguuagcuL96	646	asCfsuuaAfcGfGfugcuCfcAfGuaucscsu	647	AGACUACUGGAGCACCCGUUAAGG	648
AD-959749.1	csasgcuCfuCfCfAfGfaggaacagcuL96	649	asCfsuucGfuUfCfGcagAfgCfagcugscsc	650	GGCAGCUGCUCACAGGAACAGAGG	651
AD-959798.1	csGsaagcUfcAfGfAfGgcccaggaauL96	652	asUfscuuCfGfCfCfucGfGfAfGcucgsgsg	653	CCCAGAGCUUCAGAGGCCGAGGAU	654
AD-959742.1	csusagagGfcAfGfCfugcuccagcuL96	655	asCfscugGfGfCfCfagcuGfCfCfuaucsgsg	656	CCCUAGAGGCAGCUGCUCACAGGA	657
AD-959897.1	gsasguccCfaGfGfUfGgcccagcuL96	658	asUfsgcuGfGfCfCfaccUfgGfGcucscsu	659	AGGAGUCCAGGUGGCCACGACG	660
AD-959864.1	asgsaccCfcAfAfGfGfagcagcuL96	661	asCfsagcGfCfAfUfCcuGfGfGcucucsg	662	CAAGACCGCCAAAGGAUGCACUGA	663
AD-959899.1	csGsaagcUfgCfCfCfagcagcccauL96	664	asUfsggcCfuGfCfGgagCfcAfCfGgsgsa	665	UCCCAAGGUGGCCACAGCAGGCCAG	666
AD-959856.1	ascsgccaCfcAfAfGfagccaggaauL96	667	asCfsuugGfCfGfGfucuuGfUfGgucsgsc	668	GCACGCCACCAAGACCGCCAAAGG	669
AD-959906.1	gsgsugacCfGfAfUfGfGcucagcuL96	670	asAfsacuGfGfAfGfcauGfGfGfucacscsa	671	UGGGUGACCGAUUGGCUUCAGUUC	672
AD-959936.1	usascugGfGfAfCfCfGuaaaggaauL96	673	asUfscuuUfaAfCfGgugCfuCfCagucagcu	674	ACUACUGGAGCACCCGUUAAGGAC	675

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-959896.1	gsgsagucCfcAfGfCfuggcccagcuL96	676	asGfscugGfGcCfCfaccuGfGfGfaccuccsug	677	CAGGAGUCCCCAGGUGGCCCCAGCA	678
AD-959893.1	gscsaggaGfhuCfCfCfagguGCCcuL96	679	asGfsggcCfCfCfCfugggAfCfUfcucgsasc	680	GUGCAGGAGUCCCCAGGUGGCCCA	681
AD-959892.1	usgscagzAfGfUfCfCfagugGCCuL96	682	asGfsgccAfCfCfUfgggaCfUfCfucgascsg	683	CGUGCAGGAGUCCCCAGGUGGCC	684
AD-959894.1	csasagagUfcCfCfAfGgugGCCcuL96	685	asUfIsgggCfCfAfCfCfugGfGfCfucucscsa	686	UGCAGGAGUCCCCAGGUGGCCAG	687
AD-959750.1	asgscugUfcCfAfGfGaacagagcuL96	688	asCfscucUfGfUfUfcucugGfGfGfaccucsgsc	689	GCAGCUGCUCCAGGAACAGAGGU	690
AD-959898.1	asgsucceAfGfUfUfGfcccagcagcuL96	691	asCfSugcUfGfGfGfcaacCfUfGfGfaccucsc	692	GGAGUCCCCAGGUGGCCACGAGG	693
AD-959895.1	asgsgagucCfcCfAfGfGfugGCCcagcuL96	694	asCfSuggcGfCfCfAfccugGfGfAfccuccucsgsc	695	GcAGGAGUCCCCAGGUGGCCACG	696

Table 4. Unmodified Sense and Antisense Strand Sequences of Apolipoprotein C3 dsRNA Agents

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM__000040.3	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM__000040.3
AD-960293.1	CUUCAGUUCUCCUGAAAGACUU	24	245-265	AAGUCUTUCAGGGAACUGAAGCC	261	243-265
AD-960288.1	GAUGGCUUCACGUUCCCGAAU	44	240-260	AUUCAGGGAACUGAAGCCAUCGG	45	238-260
AD-960445.1	AAGGGACAGUAUUCUCAGUGU	46	437-457	ACACUGAGAAUACUGUCCCUUUU	47	435-457
AD-960292.1	GCUUCAGUUCUCCUGAAAGACU	42	244-264	AGUCUUTCAGGGAACUGAAGCCA	262	242-264
AD-960475.1	CCAAUAAAGCUGGACAAAGAAU	28	506-526	AUUCUUGUCCAGCUUUUAUUGGGA	29	504-526
AD-960442.1	UAAAAAGGGACAGUAUUCUCAU	36	434-454	AUGAGAAUAACUGUCCCUUUUAAG	37	432-454
AD-960470.1	GCCUCCCAAUAAAGCUGGACU	95	501-521	AGUCCAGCUUUUAUUGGGAGGCCA	96	499-521
AD-960436.1	GUUGCUUAAAGGGACAGUAU	263	428-448	AUACUGTCCCUUUUAAAGCAACCU	264	426-448
AD-960446.1	AGGGACAGUAUUCUCAGUGCU	85	438-458	AGCACUGAGAAUACUGUCCCUUU	86	436-458
AD-960474.1	CCCAAUAAAGCUGGACAAAGAU	59	505-525	AUCUUGTCCAGCUUUUAUUGGAG	265	503-525
AD-960294.1	UUCAGUUCUCCUGAAAGACUAU	26	246-266	AUAGUCTUUCAGGGAACUGAAGC	266	244-266
AD-960471.1	CCUCCCAAUAAAGCUGGACAU	54	502-522	AUGUCCAGCUUUUAUUGGGAGGCC	55	500-522
AD-960314.1	CUGGAGCACCGUUAAAGGACAU	61	266-286	AUGUCCUUAACGGUGUCCAGUA	267	264-286
AD-960443.1	AAAAGGGACAGUAUUCUCAGU	30	435-455	ACUGAGAAUACUGUCCCUUUUAA	31	433-455
AD-960282.1	GUGACCGAUGGCUUCAGUUCU	65	234-254	AGAAUCUGAAGCCAUCGGUACCCC	66	232-254

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000040.3	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000040.3
AD-960283.1	UGACCGAUGGCUUCACAGUUCU	77	235-255	AGGAACGTGAAGCCAUCCGGUCACC	268	233-255
AD-80794.7	CUUAAAAGGGACAGUAUUCUA	13	432-450	UAGAAUACUGUCCUUUUUAAAGCA	58	430-450
AD-960295.1	UCAGUCCCUAGAAAGACUACU	71	247-267	AGUAGUCUUUACAGGGAACUGAAG	72	245-267
AD-960478.1	AUAAAAGCUGGACAAAGAGCUU	89	509-529	AAGCUUCUUGUCCAGCUUUUAUUG	90	507-529
AD-960289.1	AUGGCUUCAGUUCUCCUGAAA	32	241-261	AUUUCAGGGAAACUGAAGCCAUCCG	33	239-261
AD-960481.1	AAGCUGGACAAAGAGCUGCUU	99	512-532	AAGCAGCUUCUUGUCCAGCUUUUA	100	510-532
AD-960318.1	AGCACCGUUAAAGGACAAAGUUU	34	270-290	AAACUUUGUCCUUAAACGGUGCUCC	35	268-290
AD-960297.1	AGUUCUCCUGAAAGACUACUGU	97	249-269	ACAGUAGUCUUUUCAGGGAAACUGA	98	247-269
AD-960477.1	AAUAAAAGCUGGACAAAGAGCU	105	508-528	AGCUUCUUGUCCAGCUUUUAUUGG	269	506-528
AD-960317.1	GAGCACCGUUAAAGGACAAAGUU	270	269-289	AACUUGTCCUUAAACGGUGCUCCA	271	267-289
AD-960476.1	CAAUAAAAGCUGGACAAAGAGU	81	507-527	ACUUCUTGUCCAGCUUUUAUUGGG	272	505-527
AD-960241.1	GACCGCCAAAGAUUCACUGAU	133	170-190	AUCAGUGCAUCCUUUGGGGUCUUU	134	168-190
AD-960480.1	AAAGCUGGACAAAGAGCUGCUU	117	511-531	AGCAGTUCUUUGUCCAGCUUUUAU	273	509-531
AD-960482.1	AGCUGGACAAAGAGCUGCUAU	127	513-533	AUAGCAGCUUCUUGUCCAGCUUUU	128	511-533
AD-80793.7	GCUUGGACAAAGAGCUGCUAUA	101	514-533	UAUAGCAGCUUCUUGUCCAGCUUU	102	512-533
AD-960107.1	CAGUUCAUCCCUAGAGGCAGU	175	6-26	ACUGGCTCUAGGGGAUGAACUGAG	274	6-26
AD-960308.1	AGACUACUGGAGCACCGUUUAU	73	260-280	AUAAACGGUGUCCAGUAAGUCUUU	74	258-280
AD-960121.1	AGGCAGCUGCUCCAGGAACAU	153	20-40	AUGUUCUUGGAGCAGCUGCCUCU	154	18-40
AD-960287.1	CGAUGGCUUCAGUUCUCCUGAU	83	239-259	AUCAGGGAAACUGAAGCCAUCCGU	84	237-259
AD-960473.1	UCCCAAUAAAGCUGGACAAAGU	67	504-524	ACUUGUCCAGCUUUUAUUGGGAGG	68	502-524
AD-960479.1	UAAAAGCUGGACAAAGAGCUGU	135	510-530	ACAGCUTCUUGUCCAGCUUUUAU	275	508-530
AD-960278.1	CUGGGUGACCGAUGGCUUCAU	79	230-250	AUGAAGCCAUCCGUAACCCAGCC	80	228-250
AD-960113.1	AUCCCUAAGAGGCAGCUGCUCU	165	12-32	AGAGCAGCUGCCUUCUAGGGGAUGA	166	10-32
AD-960472.1	CUCCCAAUAAAGCUGGACAAU	50	503-523	AUUGUCCAGCUUUUAUUGGGAGGC	51	501-523
AD-960444.1	AAAGGGACAGUAUUCACAGUU	63	436-456	AACUGAGAAUACUGUCCCUUUUA	64	434-456
AD-960303.1	CUGAAAGACUACUGGAGCACU	87	255-275	AGUGCUCCAGUAUCUUCUACGGG	88	253-275

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000040.3	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000040.3
AD-960438.1	UGC U U A A A A G G G A C A G U A U U U	52	430-450	A A A U A C T G U C C C U U U U A A G C A A C	276	428-450
AD-960290.1	U G G C U U C A G U U C C C U G A A A G U	93	242-262	A C U U U C A G G G A A C U G A A G C C A U C	94	240-262
AD-960304.1	U G A A A G A C U A C U G G A G C A C C U	167	256-276	A G G U G T C C A G U A G U C U U U C A G G	277	254-276
AD-960388.1	C U G C C U G A G A C C U C A A U A C C U	115	340-360	A G G U A U T G A G G U C U C A G G C A G C C	278	338-360
AD-960233.1	G C C A C C A A G A C C G C C A A G G A U	195	162-182	A U C C U U G G C G G U C U U G G U G G C G U	196	160-182
AD-960234.1	C C A C C A A G A C C G C C A A G G A U U	75	163-183	A A U C C U T G G C G G U C U U G G U G G C G	279	161-183
AD-960114.1	U C C C U A G A G G C A G C U G C U C C U	141	13-33	A G G A G C A G C U G C C U C U A G G G A U G	142	11-33
AD-960296.1	C A G U U C C C U G A A A G A C U A C U U	109	248-268	A A G U A G T C U U U C A G G G A A C U G A A	280	246-268
AD-960431.1	U G U A G G U U G C U U A A A A G G G A U	149	423-443	A U C C C U T U U A A G C A A C C U A C A G G	281	421-443
AD-960316.1	G G A G C A C C G U U A A A G G A C A A G U	187	268-288	A C U U G U C C U A A A C G G U G C U C C A G	188	266-288
AD-960307.1	A A G A C U A C U G G A G C A C C G U U U	91	259-279	A A A C G G T G C U C C A G U A G U C U U U C	282	257-279
AD-960120.1	G A G G C A G C U G C U C C A G G A A C U	181	19-39	A G U U C T T G G A G A C A G C U G C C U C A	283	17-39
AD-960238.1	C A A G A C C G C C A A G G A U G C A C U	284	167-187	A G U G C A T C C U U G G C G G U C U U G G U	285	165-187
AD-960301.1	C C C U G A A A G A C U A C U G G A G C U	103	253-273	A G C U C C A G U A G U C U U U C A G G G A A	104	251-273
AD-960235.1	C A C C A A G A C C G C C A A G G A U G U	123	164-184	A C A U C C T U G G C G G U C U U G G U G G C	286	162-184
AD-960123.1	G C A G C U G C U C C A G G A A C A G A U	155	22-42	A U C U G U T C C U G G A G C A G C U G C C U	287	20-42
AD-960300.1	U C C C U G A A A G A C U A C U G G A G U	137	252-272	A C U C C A G U A G U C U U U C A G G G A A C	138	250-272
AD-960285.1	A C C G A U G G C U U C A G U U C C C U U	38	237-257	A A G G G A A C U G A A A G C C A U C G G U C A	39	235-257
AD-960469.1	G G C C U C C C A A U A A A A G C U G G A U	173	500-520	A U C C A G C U U U A U U G G G A G G C C A G	174	498-520
AD-960387.1	G C U G C C U G A G A C C U C A A U A C U	171	339-359	A G U A U U G A G G U C U C A G G C A G C C A	172	337-359
AD-960384.1	G U G G C U G C C U G A G A C C U C A A U	183	336-356	A U U G A G G U C U C A G G C A G C C A C G G	184	334-356
AD-960109.1	G U U C A U C C C U A G A G G C A G C U U	225	10-28	A A G C U G C C U C U A G G G A U G A A C U G	226	6-28
AD-960112.1	C A U C C C U A G A G G C A G C U G C U U	113	11-31	A A G C A G C U G C C U C U A G G G A U G A A	114	9-31
AD-960386.1	G G C U G C C U G A G A C C U C A A U A U	177	338-358	A U A U U A G G U C U C A G G C A G C C A C	178	336-358
AD-960302.1	C C U G A A A G A C U A C U G G A G C A U	111	254-274	A U G C U C C A G U A G U C U U U C A G G G A	112	252-274
AD-960118.1	U A G A G G C A G C U G C U C C A G G A U	221	17-37	A U C C U G G A G C A G C U G C C C U C A G G	222	15-37

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000040.3	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000040.3
AD-960111.1	UCAUCCCUAGAGGCAGCUGCU	199	10-30	AGCAGCTGCCUCUAGGGAUGAAC	288	8-30
AD-960299.1	UUCCCUAGAAAGACUACUGGAU	119	251-271	AUCCAGTAGUCUUUCAGGGAAACU	289	249-271
AD-960115.1	CCCUAGAGGCAGCUGCUCCAU	129	14-34	AUGGAGCAGCUGCCUCUAGGGAU	130	12-34
AD-960439.1	GCUUAAAAAGGGACAGUAUUCU	56	431-451	AGAAUACUGUCCCUUUUAAAGCAA	57	429-451
AD-960441.1	UUAAAAAGGGACAGUAUUCUCU	40	433-453	AGAGAAATACUGUCCCUUUUAAAGC	290	431-453
AD-960232.1	CGCCACCAAGACCGCCAAGGU	157	161-181	ACCUUGGCGGUCUUGGUGGCGUG	158	159-181
AD-960276.1	GGCUGGGUGACCGAUUGGCUUU	107	228-248	AAAGCCAUCGGUCACCCAGCCCC	108	226-248
AD-960435.1	GGUUGCUUAAAAAGGGACAGUU	69	427-447	AACUGUCCCUUUUAAAGCAACCUA	70	425-447
AD-960306.1	AAAGACUACUGGAGCACCGUU	151	258-278	AACGGUGUCCAGUAGUCUUUCA	152	256-278
AD-960172.1	CCGAGCUUCAGAGGCCGAGGU	217	101-121	ACCUCGGCCUCUGAAAGCUCGGGC	218	99-121
AD-960385.1	UGGCUGCCUGAGACCUCUAAUU	161	337-357	AAUUGAGGUUCUAGGCAAGCCACG	162	335-357
AD-960110.1	UUCAUCCCUAGAGGCAGCUGU	201	9-29	ACAGCUGCCUCUAGGGAUGAACU	202	7-29
AD-960116.1	CCUAGAGGCAGCUGCUCCAGU	147	15-35	ACUGGAGCAGCUGCCUCUAGGGA	148	13-35
AD-960434.1	AGGUUGCUUAAAAAGGGACAGU	125	426-446	ACUGUCCCUUUUAAAGCAACCUAC	126	424-446
AD-960430.1	CUGUAGGUUGCUUAAAAAGGGU	189	422-442	ACCCUUTUAAAGCAACCUACAGGG	291	420-442
AD-960305.1	GAAAGACUACUGGAGCACCGU	179	257-277	ACGGUGGUCCAGUAGUCUUUCAG	180	255-277
AD-960279.1	UGGGUGACCGAUUGGCUUCAGU	215	231-251	ACUGAAAGCCAUCCGGUCACCCAGC	216	229-251
AD-960298.1	GUUCCCUGAAAGACUACUGGU	191	250-270	ACCAGUAGUCUUUCAGGGAAACUG	192	248-270
AD-960284.1	GACCGAUGGCUUCAGUUCCCU	139	236-256	AGGGAACUGAAAGCCAUCGGUCAC	140	234-256
AD-960313.1	ACUGGAGCACCGUUUAAAGGACU	213	265-285	AGUCCUTAAACGGUGCUCUCCAGUAG	292	263-285
AD-960432.1	GUAGGUUGCUUAAAAAGGGACU	145	424-444	AGUCCCTUUUAAAGCAACCUACAG	293	422-444
AD-960124.1	CAGCUGCUCCAGGAACAGAGU	229	23-43	ACUCUGTUCUCCUGGAGCAGCUGCC	294	21-43
AD-960119.1	AGAGGCAGCUGCUCCAGGAAU	197	18-38	AUUCUGGAGCAGCUGCCUUCUAG	198	16-38
AD-960437.1	UUGCUUAAAAAGGGACAGUAUU	295	429-449	AAUACUGUCCCUUUUAAAGCAACC	296	427-449
AD-960315.1	UGGAGCACCGUUUAAAGGACAAU	131	267-287	AUUGUCCUUUAAACGGUGCUCCAGU	132	265-287
AD-960117.1	CUAGAGGCAGCUGCUCCAGGU	233	16-36	ACCUGGAGCAGCUGCCUUCUAGGG	234	14-36

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000040.3	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000040.3
AD-960311.1	CUACUGGAGCACCGUUAAGGU	159	263-283	ACCUUAACGGUGCUCCAGUAGUC	160	261-283
AD-960272.1	GAGUCCAGGUGGCCACAGCAU	235	201-221	AUGUGGGCCACCUGGGACUCCU	236	199-221
AD-960414.1	CGAGCUCCUUGGGUCCUGCAU	203	386-406	AUGCAGGACCCAAAGGAGCUCGCA	204	384-406
AD-960240.1	AGACCGCCAAAGGAUGCACUGU	237	169-189	ACAGUGCAUCCUUGCGGUCUUG	238	167-189
AD-960286.1	CCGAUGGCUUCAGUCCUGU	143	238-258	ACAGGGAACUGAAGCCAUCCGGUC	144	236-258
AD-960281.1	GGUGACCGAUUGGCUUCAGUUU	243	233-253	AAACUGAAGCCAUCGGUACACCA	244	231-253
AD-960277.1	GCUGGGUGACCGAUUGGCUUCU	185	229-249	AGAAAGCCAUCGGUACCCAGCCC	186	227-249
AD-960274.1	CCAGGUGGCCACAGAGGCCAU	239	206-226	AUGGCCCTGCUGGGCCACCUGGGA	297	204-226
AD-960108.1	AGUUCAUCCCUAGAGGCAGCU	211	11-27	AGCUGCCUCUAGGGAUGAACUGA	212	11-27
AD-960239.1	AAGACCGCCAAAGGAUGCACUU	169	168-188	AAGUGCAUCCUUGGCGGUCUUGG	170	166-188
AD-960122.1	GGCAGCUGCUCACAGGAACAGU	205	21-41	ACUGUCCUUGGAGCAGCUGCCUC	206	19-41
AD-960291.1	GGCUUCAGUUCUCCUGAAAGAU	163	243-263	AUCUUCAGGGAAACUGAAGCCAU	164	241-263
AD-960125.1	AGCUGCUCCAGGAACAGAGGU	255	24-44	ACCUCUGUUCUGGAGCAGCUGC	256	22-44
AD-960231.1	ACGCCACCAAGACCGCCAAAGU	241	160-180	ACUUGGCGGUCUUGGUGGCGUGC	242	158-180
AD-960275.1	GGGCUUGGUGACCGAUUGGCUU	193	227-247	AAGCCATCGGUCACCCAGCCCCU	298	225-247
AD-960173.1	CGAGCUUCAGAGGCCGAGGAU	231	102-122	AUCCUGGCCUCUGAAGCUCGGG	232	100-122
AD-960271.1	GGAGUCCCAAGGUGGCCACAGCU	247	200-220	AGCUGGGCCACCUGGGACUCCUG	248	198-220
AD-960433.1	UAGGUUGCUUAAAAGGGACAU	121	425-445	AUGUCCCUUUUAAAGCAACCUACA	122	423-445
AD-960267.1	UGCAGGAGUCCCAAGGUGGCCU	251	196-216	AGGCCACCUGGGACUCCUGCACG	252	194-216
AD-960236.1	ACCAAGACCGCCAAAGGAUGCU	219	165-185	AGCAUCCUUGGCGGUCUUGGGUGG	220	163-185
AD-960310.1	ACUACUGGAGCACCGUUUAAAGU	227	262-282	ACUUAAACGGUGUCUCCAGUAGUCU	228	260-282
AD-960312.1	UACUGGAGCACCGUUUAAAGGAU	245	264-284	AUCCUUAAACGGUGUCUCCAGUAGU	246	262-284
AD-960309.1	GACUACUGGAGCACCGUUUAAU	209	261-281	AUUAAACGGUGUCUCCAGUAGUCUU	210	259-281
AD-960440.1	CUUAAAAAGGGACAGUAUUCUU	48	432-452	AAGAAUACUGUCCCUUUUAAAGCA	49	430-452
AD-960237.1	CCAAGACCGCCAAAGGAUGCAU	207	166-186	AUGCAUCCUUGGCGGUCUUGGUG	208	164-186
AD-960268.1	GCAGGAGUCCCAAGGUGGCCCU	249	197-217	AGGGCCACCUGGGACUCCUGCAC	250	195-217

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000040.3	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000040.3
AD-960269.1	CAGGAGUCCACGUGGCCCAU	253	198-218	AUGGGCCACCUUGGGACUCCUGCA	254	196-218
AD-960280.1	GGGUGACCGAUUGGCUUCAGUU	223	232-252	AACUGAAGCAUCGGUACCCAG	224	230-252
AD-960270.1	AGGAGUCCACGUGGCCACAGU	259	199-219	ACUGGGCCACCUUGGGACUCCUGC	260	197-219
AD-960273.1	AGUCCACGUGGCCACGACAGU	257	202-222	ACUGGUGGCCACCUUGGGACUCC	258	200-222

Table 5. Modified Sense and Antisense Strand Sequences of Apolipoprotein C3 dsRNA Agents

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-960293.1	csusucagUfuCfCfCfugaagacuuL96	341	asAfsaguc(Tgn)ucaggGfAfaCfugaagcsc	697	GGCUUCAGUUCUCCUGAAAAGACUA	343
AD-960288.1	ususcagUfuCfCfUfgaagacuuL96	344	asUfsucag(Ggn)gaacugAfaGfccaucsgsg	698	CCGAUGGCUUCAGUUCUCCUGAAA	373
AD-960445.1	cscsaauaAfaGfCfUfggacagaauL96	347	asCfsacug(Agn)gaauacUfgUfcccususu	699	AAAAGGGACAGUAUUCUCAGUGC	376
AD-960292.1	asasaaggGfaCfAfGfuauucucaguL96	350	asGfsucuu(Tgn)caggGfaAfcUfgaagcscsa	700	UGGCUUCAGUUCUCCUGAAAAGACU	370
AD-960475.1	asusggcuUfuCfAfGfUfuccugaaauL96	353	asUfsucuu(Ggn)uccagCfUfUfaunggsa	701	UCCCAAUAAAAGCUGGACAAAGAAG	349
AD-960442.1	asgscaccGfuUfAfAfAfggacagaauL96	356	asUfsgaga(Agn)uacugCfCfhuuuuasag	702	CUUAAAAGGGACAGUAUUCUCAG	361
AD-960470.1	usasaaggGfGfAfCfAfguauucucuuL96	359	asGfsucaa(Ggn)cuuuauUfgGfaggcscsa	703	UGGCCUCCCAAUAAAAGCUGGACA	450
AD-960436.1	ascscgauGfGfCfUfUfaguuuccuuL96	362	asUfsacug(Tgn)ccuuuUfaAfgcaacscsu	704	AGGUUGCUUAAAAGGGACAGUAU	705
AD-960446.1	ususaanaGfGfAfCfAfaguuuucuuL96	365	asGfscacu(Ggn)agaauCfUfUfuccususu	706	AAAAGGGACAGUAUUCUCAGUGC	435
AD-960474.1	gscsuucaGfuUfCfCfCfugaagacuuL96	368	asUfscuug(Tgn)ccageuUfuAfuugggsag	707	CUCCCAAUAAAAGCUGGACAAAGAA	396
AD-960294.1	gsasuggcUfuCfAfGfUfuccugaaauL96	371	asUfsaguc(Tgn)uucaggGfaAfcugaagcsc	708	GCUUCAGUUCUCCUGAAAAGACUAC	346
AD-960471.1	asasgggaCfaGfUfAfUfucucaguuL96	374	asUfsgucc(Agn)gcuuuUfuGfagggscc	709	GGCCUCCCAAUAAAAGCUGGACAA	388
AD-960314.1	csusuaaaAfgGfGfAfcaguuuucuuL96	377	asUfsgucc(Tgn)uaaeggUfgCfuccagsusa	710	UACUGGAGCACCGUUUAAAGGACAA	399
AD-960443.1	csuscccaAfuAfAfAfgcuggacaauL96	380	asCfsugag(Agn)auacugUfcCfhuuuusasa	711	UUAAAAGGGACAGUAUUCUCAGU	352
AD-960282.1	usgscuuuAfaAfGfGfagacaguuuL96	383	asGfsaacu(Ggn)aaagccaUfcGfuccacscsc	712	GGGUGACCGAUUGGCUUCAGUUC	405
AD-960283.1	cscsuuccAfuUfAfAfagucggacuuL96	386	asGfsgaac(Tgn)gaagccAfuCfignucacsc	713	GGUGACCGAUUGGCUUCAGUCCCC	423
AD-80794.7	gscsuuaaAfaGfGfGfagacaguuuL96	389	usAfsagaaUfaCfUfguccCfUfuuuagcscsa	392	UAGAAUACUGUCCCUUUUUAAGCA	714
AD-960295.1	csusucagUfuCfCfCfugaagacuuL96	341	asGfsuagu(Cgn)uuucagGfGfAfaugagasag	715	CUUCAGUUCUCCUGAAAAGACUACU	414

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-960478.1	gsasuggcUfuCfAfGfuucccugaauL96	371	asAfsccuu(Cgn)luuguccAfGcfhuuausug	716	CAAUAAAAGCUGGACAAAGAACGUCG	441
AD-960289.1	asasgggaCfaGfUfAfhuucacagugL96	374	asUfsumca(Ggn)ggaaCuGfaAfGCCauscg	717	CGAUGGCUUACAGUUCUCCUGAAAG	355
AD-960481.1	gscsuucaGfuUFfCFcfeguagaacuL96	368	asAfgscag(Cgn)luucuuUfcCfiaguususa	718	UAAGAUGCUGGACAAGAAAGCUGCUA	456
AD-960318.1	cscsaauaFafGfCfUffggacagaauL96	347	asAfsacuu(Ggn)luccuuAfcGfigucuscsc	719	GGAGCACCGUUUAAAGGACAAAGUUC	358
AD-960297.1	usasaaagGfgAFcFAfguanucauL96	359	asCfsagua(Ggn)lucnuuCfGfggaucsasa	720	UCAGUUCUCCUGAAAGACUACUGG	453
AD-960477.1	gscsucceCfaAfUfAfagcuugacuL96	448	asGfscuuc(Tgn)juguceaGfcUfuuausgsg	721	CCAUAUAAAGCUGGACAAAGAACGU	465
AD-960317.1	gsusugcuUfaAfAfAfgggacagauL96	722	asAfsccuug(Tgn)cenuuaCfGfngucucsa	723	UGGAGCACCGUUUAAAGGACAAGUU	724
AD-960476.1	asgsggacAfGfUfAfufucucagucL96	433	asCfsuucu(Tgn)guccagCfuUfuuausgsg	725	CCCAAUAAAAGCUGGACAAAGAACG	429
AD-960241.1	cscscsauAfaAfGfChuggacaaguL96	394	asUfscagu(Ggn)cauccUfGfGfggucsuu	726	AAGACCGCCAAGGAUUGCACUGAG	507
AD-960480.1	ususcaguUfcCfCfUfgaagacuauL96	344	asGfscagc(Tgn)luuguuGfcAfGcuuusasu	727	AUAAAAGCUGGACAAAGAACGUCU	483
AD-960482.1	cscsucceAfaUfAfAfagcuggacuL96	386	asUfsgaac(Ggn)cunuuGfuCfcagcususu	728	AAAGCUGGACAAAGAACGUCUAU	498
AD-80793.7	csusggagCfaCfCfGhuuaaggacuL96	397	usAfsuagCfaGfCfhuucUfGfUfccagsusu	458	UAUAGCAGCUUCUUGUCCAGCUU	729
AD-960107.1	asasaagGfaCfAfGhuuuncacuL96	350	asCfsgucc(Tgn)cuaaggAfUfGaaucgsag	730	CUCAGUUAUCCCCUAGAGGCAGC	570
AD-960308.1	gsusgccGfaUfGfGfemucaguucL96	403	asUfsgaag(Cgn)juguceaAfGfUfaguususu	731	AAAGACUACUGGAGCACC GUUAA	417
AD-960121.1	usgsaccgAfUfGfGfCfuicaguucL96	421	asUfsguue(Cgn)juggagcAfGcfugccucsu	732	AGAGGCAGCUGCUCCAGGAACAG	537
AD-960287.1	csushaaaAfGfGfAfcaanuucuaL96	17	asUfscagg(Ggn)aacugaAfGcfcaucgsgsu	733	ACCGAUGGCUUCAGUUCUCCUGAA	432
AD-960473.1	uscsaguuCfcCfUfCfaaagacuacL96	412	asCfsumgu(Cgn)cagcuUfaUfugggagsgsg	734	CCUCCCAAUAAAGCUGGACAAGA	408
AD-960479.1	asusaaagCfuGfGfAfcaagaagcuL96	439	asCfsagcu(Tgn)cuuugcCfiaGfumuususu	735	AUAUAAAGCUGGACAAAGAACGUC	510
AD-960278.1	asusggcuUfcAfGfUfucccugaauL96	353	asUfsgaig(Cgn)caucggUfcAfcccagcsc	736	GGCUGGGUGACCGGAUGGCUUCAG	426
AD-960113.1	asasgcugGfaCfAfAfzaagcugcuL96	454	asGfsagca(Ggn)cugccuCfuAfgggauksa	737	UCAUCCCUAGAGGCAGCUGCUCC	555
AD-960472.1	asgsaccGfuUfAfAfzgacaagnuL96	356	asUfsguuc(Cgn)agcuuAfUfUfgggagsgsc	738	GCCUCCCAAUAAAGCUGGACAAG	382
AD-960444.1	asgsnuuceCfuGfAfAfagacuacugL96	451	asAfsccuig(Ggn)aaucuGfuCfcuuususa	739	UAAAAGGGACAGUAUUCUCAGUG	402
AD-960303.1	asashaaaGfcUfGfGfGfacagaagcuL96	463	asGfsgucU(Cgn)caguagUfcUfuucagsgsg	740	CCCUGAAAGACUACUGGAGCACACC	438
AD-960438.1	gsasgcacCfGfUfUfAfagcacaguL96	741	asAfsanac(Tgn)gucccuUfuUfaagcasasc	742	GUUGCUUAAAAAGGACACAGUAUUC	385
AD-960290.1	csasauaaAfGfUfUfAfagcacaguL96	427	asCfsuunc(Agn)eggnaeUfGfAgaccasusc	743	GAUGGCUUCAGUUCUCCUGAAAGA	447
AD-960304.1	gsasccgcCfaAfGfGfGaugcacugL96	505	asGfsguge(Tgn)ccaguaGfuCfuucacsugsg	744	CCUGAAAAGACUACUGGAGCACCG	558

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-960388.1	asasagcuGfgAfCfAfagaagcugcuL96	481	asGfsguau(Tgn)gaggucUfcAfzgagcscc	745	GGCUGCCUGAGACCUCUCAAUACCC	480
AD-960233.1	asgscugGfCfAfGfagcugcuauL96	496	asUfscuu(Cgn)ggcucUfuGfuggsesu	746	AGCCACCAAGACCGCCAAAGGAU	600
AD-960234.1	gscsugGfCfAfGfAfagcugcuauL96	457	asAfscuu(Tgn)ggcggUfUfuggsesu	747	CGCCACCAAGACCGCCAAAGGAU	420
AD-960114.1	csasgucAfUfCfCfCfagagcagL96	568	asGfsgagc(Agn)gcugccUfcUfaggasusg	748	CAUCCCUAGAGGCAGCUGCUCCA	519
AD-960296.1	asgsacuaCfuGfGfAfagcagcuauL96	415	asAfsguag(Tgn)cuuuaGfgGfagcuasasa	749	UUCAGUCCCUUGAAAGACUACUG	471
AD-960431.1	asgsgcagCfuGfCfUfcaggaacauL96	535	asUfscuu(Tgn)uuuagcAfaCfcauacsag	750	CCUGUAGGUUGCUUAAAAGGGAC	531
AD-960316.1	csgsaugGfUfCfAfGfugccuauL96	430	asCfscuu(Cgn)cuuuaGfgUfgcuccsag	751	CUGGAGCACCGUUUAAAGGACAAAGU	588
AD-960307.1	uscscaaUfaAfAfGfGfagcagcuL96	406	asAfscag(Tgn)gcuccaGfuAfGcuuisc	752	GAAAGACUACUGGAGCACCCGUUA	444
AD-960120.1	usasaaGfUfGfAfCfAfaagagcuL96	508	asGfscuu(Cgn)ggagcGfCfUfgcuccusa	753	UAGAGGCAGCUGCUCCAGGAACA	579
AD-960238.1	csusggGfCfCfGfGfagggcuauL96	424	asGfsguag(Tgn)ccuugGfGfGfuggsesu	754	ACCAAGACCGCCAAAGGAUGCACU	755
AD-960301.1	asusccuAfGfAfGfGfagcugcuL96	553	asGfscucc(Agn)guagUfuUfagggasasa	756	UUCCCUGAAAGACUACUGGAGCA	462
AD-960235.1	csuscccaAfUfAfAfGfGfagcagcuL96	380	asCfscucc(Tgn)ggcggUfcUfuguggscc	757	GCCACCAAGACCGCCAAAGGAUGC	492
AD-960123.1	asasaggGfCfAfGfUfaucucaguuL96	400	asUfscuu(Tgn)ccuugGfCfAfGfugcsesu	758	AGGACGUGCUCCAGGAACAGAG	540
AD-960300.1	csusgaaGfCfUfAfGfGfagcagcuL96	436	asCfscucc(Ggn)uagucUfuCfagggasasc	759	GUUCCCUGAAAGACUACUGGAGC	513
AD-960285.1	usgsccuaAfAfGfGfagcagcuauL96	383	asAfsggga(Agn)cuuagCfCfAfucgusasa	760	UGACCGAUGGCUUACAGUCCCUUG	364
AD-960469.1	usgsgcuCfAfGfUfUfcccugaaagL96	445	asUfscag(Cgn)uuuuGfGfGfagggcsasg	761	CUGGCCUCCCAAUAAAGCUGGAC	567
AD-960387.1	usgsaaagAfCfUfAfCfGfagcagcuL96	556	asGfscuu(Cgn)aggucUfaGfGfagcsasa	762	UGGCGCCUGAGAGACCUCUCAAUACC	564
AD-960384.1	csusgcuGfAfGfAfCfCfcauauccuL96	478	asUfsgag(Cgn)ucucagGfCfAfccacsgsg	763	CCGUGGCGCCUGAGACCUCUCAAU	582
AD-960109.1	gscsaccAfAfGfAfCfCfccaaggaL96	598	asAfsgcag(Cgn)cuuagGfGfAfugaacsusg	764	CAGUUAUCCCUAGAGGCGAGCUG	645
AD-960112.1	csccsacAfGfAfCfCfGfccaaggaL96	418	asAfsgcag(Cgn)ggcucUfaGfGfaggsasa	765	UUCAUCCCUAGAGGCGAGCUGCUC	477
AD-960386.1	uscsccuGfAfGfGfGfagcugcuL96	517	asUfscuu(Cgn)ggcucUfaGfGfaggsasa	766	GUGGCGCCUGAGACCUCUCAAUAC	573
AD-960302.1	csasgucCfCfUfGfAfagcagcuL96	469	asUfsgcag(Cgn)uagucUfuUfagggasa	767	UCCCUGAAAGACUACUGGAGCAC	474
AD-960118.1	usgsuaggUfUfGfCfUfaaaagggaL96	529	asUfscuu(Cgn)ggcucUfaGfGfaggsasa	768	CCUAGAGGCAGCUGCUCCAGGAA	639
AD-960111.1	gsgsagcaCfCfUfUfaaggagcuL96	586	asGfscag(Cgn)ggcucUfaGfGfaggsasa	769	GUUCAUCCCUAGAGGCGAGCUGCU	606
AD-960299.1	asasgcuAfCfUfGfGfagcagcuL96	442	asUfscag(Cgn)uagucUfaGfGfaggsasa	770	AGUCCCUGAAAGACUACUGGAG	486
AD-960115.1	gsasgcaGfCfUfGfCfuccaggaL96	577	asUfsggag(Cgn)aggucCfuCfuaagggsasu	771	AUCCCUAGAGGCGAGCUGCUCCAG	501

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-960439.1	csasagacCfGcCfAfaggaugcacuL96	772	asGfsaaua(Cgn)ugucccUfuUfuuaacsasa	773	UUGCUUAAAAGGGACAGUAUUCU	391
AD-960441.1	cscscugaAfaGfAfCfuacugagcuL96	460	asGfsagaa(Tgn)acugucCfcUfuuaasgsc	774	GCUAAAAGGGACAGUAUUCUCA	367
AD-960232.1	csasccaaGfaCfCfGfccaaggaugL96	490	asCfscuug(Cgn)cgguuUfGfuggggsug	775	CACGCCACCAAGACC GCCCAAGGA	543
AD-960276.1	gscsagcuGfcUfCfCfaggaacagauL96	538	asAfsagcc(Agn)ucggucAfcCfcagccsc	776	GGGGCUGGGUGACCGAUGGCUUC	468
AD-960435.1	uscscugAfaGfAfCfuacugagcuL96	511	asAfsicug(Cgn)ccuuuAfaGfcaaccusa	777	UAGGUUGCUUAAAAGGGACAGUA	411
AD-960306.1	ascscgauGfGcCfUfUfCfagucccuL96	362	asAfsccgu(Ggn)uccagUfaGfucuuusca	778	UGAAAAGACUACUGGAGCACCGUU	534
AD-960172.1	gsgscucCfcAfAfUfaaagcugauL96	565	asCfscug(Cgn)ccucugAfaGfucgggsc	779	GCCCGAGCUUCAGAGGCCGAGGA	633
AD-960385.1	gscsugccUfGfAfGfAfcuacauL96	562	asAfsuuga(Ggn)gucuaGfGfagccscsg	780	CGUGGCUGCCUGAGACCUCUAAUA	549
AD-960110.1	gsusggcuGfcCfUfGfagaccuauL96	580	asCfsagcu(Ggn)ccuuaGfGfauagaacsu	781	AGUUAUCCCUAGAGGCAGCUGC	609
AD-960116.1	gsuscauCfcCfUfAfgagcagcuL96	643	asCfsugaa(Ggn)ccagucCfcUfcaaggsga	782	UCCCUAGAGGCAGCUGCUCCAGG	528
AD-960434.1	csasuccUfaGfAfGfGfagcugcuL96	475	asCfsuguc(Cgn)ccuuuAfcCfcaaccasc	783	GUAGGUUGCUUAAAAGGGACAGU	495
AD-960430.1	gsgscugCfuGfAfGfAfcuacauL96	571	asCfscu(Cgn)uagaaAfcCfuaagsgsg	784	CCCUGUAGGUUGCUUAAAAGGGA	591
AD-960305.1	cscsugaaAfgAfCfUfagcagcuL96	472	asCfsggug(Cgn)uccagUfGfucuuusag	785	CUGAAAAGACUACUGGAGCACCCGU	576
AD-960279.1	usasgagCfaGfCfUfGfuccaggaL96	637	asCfsguua(Ggn)ccaucGfuCfaccasgsc	786	GCUGGGUGACCCG AUGGCUUCAGU	630
AD-960298.1	uscsauccCfuAfGfAfgcagcugcuL96	604	asCfscagu(Agn)gucuuCfaGfaggacsuug	787	CAGUCCCUUGAAAAGACUACUGGA	594
AD-960284.1	ususcccuGfaAfAfGfAfcuacugauL96	484	asGfsggaa(Cgn)ugaaGcCfaUfGfugucasc	788	GUGACCGAUGGCUUCAGUUCUCCU	516
AD-960313.1	cscscuagAfgGfCfAfgcugcuccauL96	499	asGfsuccu(Tgn)aacggUfCfaccagusaag	789	CUACUGGAGCACCCGUAAGGACA	627
AD-960432.1	gscsuuaaAfaGfGfGfagcuaucuuL96	389	asGfsuccu(Tgn)uuuaagCfaAfcuacasc	790	CUGUAGGUUGCUUAAAAGGGACA	525
AD-960124.1	ususaaaaGfGfAfCfaguuuucuuL96	365	asCfscuug(Tgn)uccugAfgCfagucscsc	791	GGCAGCUGCUCCAGGAACAGAGG	651
AD-960119.1	csgscacCfaAfGfAfccgcaagcuL96	541	asUfsuccu(Cgn)gagcagCfuGfcccusag	792	CUAGAGGCAGCUGCUCCAGGAAC	603
AD-960437.1	gsgscugGfuGfAfCfagcugcuuL96	466	asAfsuacu(Ggn)uccuuUfuAfcagaaasc	793	GGUUGCUUAAAAGGGACAGUAUU	794
AD-960315.1	gsgsuugUfuAfAfAfcaggaacuuL96	409	asUfsuguc(Cgn)uuuagGfuGfuccacgsu	795	ACUGGAGCACCCGUUAAAAGGACAAG	504
AD-960117.1	asasagacUfaCfUfGfagcaccguL96	532	asCfscug(Agn)gcagucGfcCfucuaagsg	796	CCCUAGAGGCAGCUGCUCCAGGA	657
AD-960311.1	cscsgagcUfuCfAfGfagccgagcuL96	631	asCfscuua(Agn)cgguuGfcCfaguuagusc	797	GACUACUGGAGCACCCGUUAAAAGGA	546
AD-960272.1	usgsgcuGfcUfGfAfGfAfcuacauL96	547	asUfsgcu(Ggn)gccaccUfGfGfagucscsu	798	AGGAGUCCAGGUGGCCAGCAG	660
AD-960414.1	ususcaucCfcUfAfGfagcagcuL96	607	asUfsgcag(Ggn)accacGfGfagucgscsa	799	UGCGAGCUCCUUGGGUCCUGCAA	612

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-960240.1	cscsuagaGfgCfAfGfcuguccagunL96	526	asCfsagug(Cgn)auccunGfgCfGgucusug	800	CAAGACCGCCAAAGGAUGCAACUGA	663
AD-960286.1	agsgsuugChuUFaFafAfaagggacagunL96	493	asCfsaggg(Agn)acugaaGfcCfaucggsuc	801	GACCGAUGGCUUCAGUUCUCCUGA	522
AD-960281.1	csusguagGfuUFGfCfuuaaagggnL96	589	asAfsacug(Agn)agecauCfGfGfuacccsa	802	UGGGUGACCGAUGGCUUCAGUUC	672
AD-960277.1	gsasaagaChuAfCfUfaggacacgnL96	574	asGfsaagc(Cgn)aucgguCfAfCfcagcscc	803	GGGCGGGGUGACCGAUGGCUUCA	585
AD-960274.1	usgsngugAfcCfGfAfuggcucagunL96	628	asUfsggcc(Tgn)gcuggGfCfAfccuggsa	804	UCCCAGGUGGCCCCAGCAGGCCAG	666
AD-960108.1	gsusuccUfGfAfAfGfacuacugunL96	592	asGfscugc(Cgn)ucuaagGfaUfgaacusgsa	805	UCAGUUCAUCCCUAGAGGCAGCU	624
AD-960239.1	gsasccgaUfGfGfCfUfcaguucccnL96	514	asAfsigugc(Agn)uuccugGfcGfgucuuusg	806	CCAAGACCGCCAAAGGAUGCACUG	561
AD-960122.1	ascsuaggAfcAfCfCfgunaaaggacunL96	625	asCfsugnu(Cgn)cuaggGfAfGfcugccsusc	807	GAGGACGUGUCUCCAGGAACAGA	615
AD-960291.1	gsusagguUfGfCfUfUfaaaggagacunL96	523	asUfscuuu(Cgn)agggaChuGfaagccsas	808	AUGGCUUCAGUUCUCCUGAAAGAC	552
AD-960125.1	csasgcChuCfCfAfGgaacagagunL96	649	asCfscuuc(Ggn)uuccugGfaGfcaugsgsc	809	GCAGCUGCUCCAGGAACAGAGGU	690
AD-960231.1	agsasagcAfGfCfUfGfuccaggaunL96	601	asCfsnugg(Cgn)ggucuuGfGfUfgggugsgc	810	GCACGCCACCAAGACCGCCAAAGG	669
AD-960275.1	ususgcuuAfAfAfGfggacaguanuL96	811	asAfsgccA(Tgn)cggucaCfCfGagccscsu	812	AGGGGUGGGUGACCGAUGGCUU	597
AD-960173.1	usgsagcAfCfGfUfuaagzacauL96	502	asUfscuuc(Ggn)gccuucGfaAfGfcugsgsg	813	CCCAGCUUCAGAGGCCGAGGAU	654
AD-960271.1	csusagGfcAfGfCfUfguccagunL96	655	asGfscuug(Ggn)ccacuGfGfGfaccusug	814	CAGGAGUCCAGGUGGCCCCAGCA	678
AD-960433.1	csusacugGfaGfCfAfccgnaaaggunL96	544	asUfsgucc(Cgn)uuuuuaaGfcAfaccuasca	815	UGUAGGUUGCUUAAAAAGGGACAG	489
AD-960267.1	gsasguccCfAgGfGfUfggccagcauL96	658	asGfsgcca(Cgn)cuaggGChuCfcaugcascg	816	CGUGCAGGAGUCCAGGUGGCCCC	684
AD-960236.1	cgsasguCfcUfUfGfgucuccagunL96	610	asGfscuuc(Cgn)uuggcgGfuCfuugusgsg	817	CCACCAAGACCCGCCAAGGAUGCA	636
AD-960310.1	agsasagCfcAfAfGfgaugcacunL96	661	asCfsuaaC(Cgn)ggugucCfCfAfguagucsu	818	AGACUACUGGAGCACCGUUUAAGG	648
AD-960312.1	cscsgaugGfcUfUfCfaguucccgunL96	520	asUfscuu(Agn)acggugChuCfcauguasgu	819	ACUACUGGAGCACCGUUUAAGGAC	675
AD-960309.1	gsgsugacCfGfAfUfGfgucucagunL96	670	asUfsgaac(Ggn)gucucCfAgfuaucsuu	820	AAGACUACUGGAGCACCGUUUAAG	621
AD-960440.1	gscsngggUfGfAfCfCfgaugccuunL96	583	asAfsgaau(Agn)cuuguccChuUfuuaagsca	821	UGCUIAAAAGGGACAGUAUUCUC	379
AD-960237.1	cscsagguGfGfCfCfCfagcagccauL96	664	asUfsgau(Cgn)cuuugcgGfgUfcuugsgug	822	CACCAAGACCGCCAAAGGAUGCAC	618
AD-960268.1	agsnuucaUfcCfCfUfagaggagcaunL96	622	asGfsggcc(Agn)ccuggGfAfUfcuugcsac	823	GUGCAGGAGUCCAGGUGGCCCA	681
AD-960269.1	asasagccCfcCfAfAfGgaugacauL96	559	asUfsgggc(Cgn)accugGfAfCfuucgsca	824	UGCAGGAGUCCAGGUGGCCAG	687
AD-960280.1	gsgsagcUfGfCfUfCfaggaacagunL96	613	asAfsicug(Agn)gccaucGfgUfcacccsasg	825	CUGGGUGACCGAUGGCUUCAGUU	642
AD-960270.1	gsgscuucAfGfUfUfCfcuagaagauL96	550	asCfsuggg(Cgn)ccaucGfGfAfccuucsgc	826	GCAGGAGUCCAGGUGGCCACGC	696

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-960273.1	asgscugcUfcCfAfGfgaacagagguL96	688	asCfsugcu(Ggm)ggccacCfuGfggacuscsc	827	GGAGUCCCCAGGUGGGCCCCAGCAGG	693

Table 6. APOC3 Single Dose Screens in Hep3B cells

	50 nM Dose		10 nM Dose		1.0 nM Dose		0.1 nM Dose	
Duplex	Avg % APOC3 mRNA Remaining	SD	Avg % APOC3 mRNA Remaining	SD	Avg % APOC3 mRNA Remaining	SD	Avg % APOC3 mRNA Remaining	SD
AD-959917.1	4.2	1.8	5.1	1.6	17.7	3.8	53.5	5.1
AD-959918.1	5.1	0.8	5.6	2.2	34.5	13.4	52.1	10.0
AD-960096.1	7.8	4.6	7.3	2.2	24.3	2.1	88.2	6.8
AD-960064.1	3.7	0.4	8.2	1.0	22.1	6.2	40.1	7.3
AD-959914.1	5.9	1.1	9.8	2.4	39.4	4.2	70.4	17.8
AD-959941.1	11.5	6.1	9.9	1.7	33.7	2.9	75.4	4.8
AD-960031.1	6.5	2.2	9.9	3.0	18.8	3.4	47.6	2.7
AD-959910.1	5.5	0.9	10.5	1.6	28.4	7.3	60.4	5.8
AD-960063.1	8.5	3.1	11.1	2.4	29.7	7.1	79.4	10.0
AD-67781.7	6.1	1.5	11.2	2.5	45.7	2.2	61.4	3.7
AD-67782.2	6.3	1.0	12.4	5.6	38.0	2.3	68.4	8.5
AD-959916.1	5.3	0.7	12.5	2.8	45.6	5.9	58.5	2.0
AD-959913.1	6.2	0.3	12.7	1.1	32.2	5.9	67.0	10.0
AD-960066.1	7.7	1.1	13.2	1.6	42.3	3.7	94.7	27.8
AD-960062.1	8.8	1.2	13.7	1.8	48.8	4.7	73.1	16.8
AD-960093.1	12.5	1.8	13.7	3.0	37.4	12.5	64.0	17.2
AD-960061.1	7.3	0.4	13.9	1.3	42.4	7.9	69.2	10.6
AD-960092.1	16.2	10.7	14.2	2.0	53.1	13.9	95.0	13.2
AD-960030.1	14.8	5.6	14.7	1.2	40.7	8.2	94.2	9.5
AD-80794.6	15.2	6.5	15.5	1.7	41.3	5.3	84.6	1.8
AD-960095.1	12.6	1.6	16.0	1.9	55.4	9.0	86.4	2.9
AD-959938.1	14.9	8.3	16.5	3.4	59.9	12.6	83.8	4.5
AD-960065.1	12.6	3.5	17.1	3.8	51.7	7.4	79.1	10.3
AD-959907.1	10.7	5.0	17.4	6.4	51.1	3.5	89.4	10.9
AD-960094.1	12.4	3.7	18.8	5.3	76.5	17.2	131.2	4.0
AD-960060.1	12.9	2.6	19.3	3.7	77.8	5.5	69.6	5.8
AD-959919.1	14.1	3.1	19.8	2.0	72.6	13.4	105.9	9.6
AD-959932.1	11.8	5.9	20.1	8.9	47.1	9.8	102.2	4.3
AD-959859.1	20.6	8.2	20.5	6.8	93.5	12.1	161.4	40.6
AD-959908.1	9.6	2.2	20.8	2.5	48.7	4.7	78.8	18.0
AD-959903.1	12.9	5.5	20.9	3.1	58.0	14.2	96.2	12.5
AD-960097.1	19.1	6.5	21.0	4.9	77.5	11.3	78.4	3.8
AD-959912.1	12.0	1.3	21.1	1.3	75.3	10.6	108.4	26.5
AD-960067.1	19.9	4.6	21.2	3.6	81.4	11.3	85.8	9.3
AD-959927.1	16.1	2.6	21.3	6.3	68.8	13.0	102.7	17.7
AD-960099.1	13.4	3.0	21.9	3.6	46.1	8.7	76.3	4.2
AD-959931.1	20.4	5.4	22.6	7.6	58.7	5.0	105.7	12.0
AD-959879.1	12.6	5.1	22.8	6.0	89.8	10.4	78.1	2.3
AD-960091.1	29.8	7.8	23.2	6.8	84.3	16.5	159.8	73.0
AD-959921.1	14.9	5.1	23.2	6.6	64.2	8.3	71.0	7.7

	50 nM Dose		10 nM Dose		1.0 nM Dose		0.1 nM Dose	
Duplex	Avg % APOC3 mRNA Remaining	SD	Avg % APOC3 mRNA Remaining	SD	Avg % APOC3 mRNA Remaining	SD	Avg % APOC3 mRNA Remaining	SD
AD-960102.1	19.2	3.9	23.3	10.3	43.4	10.5	75.9	13.5
AD-80793.6	21.8	3.6	24.3	3.9	51.8	12.8	90.4	7.2
AD-959925.1	13.0	1.5	25.2	3.3	75.6	10.3	87.4	21.4
AD-960098.1	31.3	4.6	26.8	6.1	103.2	15.8	93.3	4.5
AD-959901.1	23.6	8.4	27.1	6.8	97.7	8.5	162.1	29.0
AD-959920.1	21.7	0.4	28.1	4.4	83.1	11.3	101.1	20.9
AD-959926.1	21.6	6.1	29.3	8.9	98.8	21.6	113.8	18.8
AD-959737.1	33.0	9.7	30.3	7.5	78.6	12.9	95.6	8.4
AD-960011.1	14.5	4.9	33.2	5.1	104.7	19.0	101.2	12.7
AD-960101.1	24.7	4.0	33.2	10.2	82.8	7.0	159.8	73.4
AD-959923.1	14.1	3.3	33.2	2.8	74.7	12.0	78.8	4.9
AD-68107.2	21.1	5.4	33.3	5.5	67.1	11.7	113.1	10.0
AD-960058.1	35.5	4.4	33.7	6.2	98.2	19.6	138.9	18.2
AD-68103.2	27.9	6.5	34.1	11.0	69.9	2.5	105.4	14.6
AD-959860.1	27.6	6.2	35.5	11.2	98.2	16.5	164.2	15.9
AD-960059.1	17.1	2.1	38.7	5.3	131.1	18.8	119.6	59.9
AD-960103.1	40.3	3.0	39.4	5.8	69.9	4.1	95.8	2.3
AD-959740.1	37.2	4.1	39.5	5.8	97.8	27.9	96.4	7.4
AD-959939.1	31.1	2.2	40.4	6.6	94.9	12.6	104.9	21.0
AD-959865.1	35.7	8.5	41.0	2.8	92.9	11.8	111.2	7.3
AD-960100.1	31.6	3.3	43.2	10.9	75.7	11.1	103.8	17.7
AD-959924.1	21.9	4.3	43.2	8.2	102.8	20.3	96.8	10.7
AD-959909.1	24.2	3.5	45.1	3.1	92.8	7.8	79.7	3.7
AD-959739.1	38.8	2.4	46.4	7.9	101.5	5.6	108.4	4.3
AD-959911.1	21.5	3.8	46.9	9.1	90.4	13.1	104.5	9.5
AD-960057.1	32.9	2.5	47.8	3.1	95.1	7.8	79.8	12.9
AD-959741.1	42.5	1.7	48.5	6.4	89.5	14.3	117.1	10.4
AD-960056.1	24.9	0.9	48.6	6.1	87.6	15.6	85.7	6.9
AD-959930.1	52.4	8.4	49.4	9.3	86.6	17.9	118.5	15.0
AD-959746.1	41.4	5.4	49.6	2.4	62.0	7.8	111.9	15.6
AD-959748.1	40.9	5.9	50.1	15.5	89.4	14.7	100.3	10.6
AD-959857.1	41.9	19.2	51.7	3.9	106.7	11.7	105.0	14.0
AD-959935.1	35.3	2.3	54.1	13.2	126.1	24.7	188.1	72.0
AD-960008.1	56.3	9.9	54.4	5.8	92.2	7.7	109.6	19.1
AD-959915.1	30.3	5.8	57.3	3.7	104.2	15.9	82.1	7.3
AD-959738.1	52.6	10.0	58.6	9.4	94.3	14.0	100.5	22.3
AD-959928.1	42.3	6.1	60.0	7.6	85.3	11.8	123.3	14.2
AD-959863.1	58.7	4.0	60.1	9.5	81.4	4.9	119.4	8.8
AD-960010.1	58.6	8.9	60.3	11.8	115.5	18.5	105.5	13.5
AD-960090.1	73.2	10.7	60.5	16.0	102.0	14.8	95.3	11.5
AD-959732.1	45.4	6.7	63.0	6.1	101.4	14.6	118.0	11.4

	50 nM Dose		10 nM Dose		1.0 nM Dose		0.1 nM Dose	
Duplex	Avg % APOC3 mRNA Remaining	SD	Avg % APOC3 mRNA Remaining	SD	Avg % APOC3 mRNA Remaining	SD	Avg % APOC3 mRNA Remaining	SD
AD-960009.1	50.6	5.9	63.1	17.5	106.1	10.9	109.4	19.6
AD-959929.1	72.2	10.3	66.2	15.5	140.7	47.3	108.3	18.1
AD-959745.1	48.8	7.7	66.8	15.0	104.7	17.4	125.3	7.3
AD-960007.1	64.1	8.4	67.0	13.1	86.3	8.9	104.1	7.6
AD-959902.1	41.4	9.8	68.2	4.7	98.8	18.0	132.1	37.4
AD-959940.1	50.8	9.8	69.0	9.1	105.0	18.3	96.8	13.0
AD-960055.1	52.7	8.5	70.1	5.7	101.4	20.6	115.3	18.2
AD-959922.1	54.7	6.2	70.1	6.4	113.6	16.0	93.0	7.3
AD-959900.1	65.2	7.0	70.6	5.3	102.9	11.3	179.9	77.2
AD-959858.1	72.9	18.5	71.4	17.7	103.4	11.1	116.6	6.1
AD-959744.1	56.0	11.2	71.6	22.1	106.2	18.3	127.0	21.8
AD-959736.1	67.3	8.2	72.6	10.6	100.5	15.8	108.3	15.6
AD-959735.1	68.8	14.9	75.5	15.3	112.0	26.5	116.0	19.0
AD-960039.1	94.8	13.8	75.5	15.3	112.7	8.6	138.0	14.7
AD-959747.1	75.4	8.4	76.0	10.3	121.7	13.3	100.0	5.8
AD-959862.1	79.3	3.4	77.9	25.8	103.2	7.9	118.8	12.4
AD-959933.1	66.7	12.1	79.0	10.7	121.8	16.7	142.3	29.2
AD-959733.1	60.8	10.8	81.7	15.8	95.7	15.4	114.2	9.7
AD-959937.1	65.7	6.0	85.2	13.6	120.8	17.1	101.8	3.2
AD-959904.1	52.7	7.6	86.9	6.4	130.2	24.2	124.9	16.7
AD-959797.1	85.6	3.9	87.2	16.3	102.0	5.9	129.5	24.1
AD-959861.1	63.0	14.0	88.4	16.5	109.2	7.3	140.1	25.0
AD-959743.1	56.2	4.8	89.3	9.4	100.1	5.8	143.9	25.4
AD-959905.1	55.9	20.6	91.7	11.8	110.6	16.2	122.3	33.1
AD-959734.1	67.0	10.9	93.5	18.4	100.7	19.0	128.9	13.4
AD-959934.1	82.1	17.1	95.1	24.1	109.8	7.7	115.6	12.4
AD-959749.1	74.1	15.9	95.7	13.1	111.2	15.8	129.2	16.1
AD-959798.1	111.9	18.4	96.6	20.7	134.4	42.1	124.5	40.5
AD-959742.1	79.3	6.8	104.1	18.5	117.5	14.2	167.7	48.7
AD-959897.1	114.4	14.2	104.7	20.1	95.8	14.8	103.7	11.1
AD-959864.1	139.0	7.4	108.7	19.7	121.8	8.3	134.4	34.1
AD-959899.1	111.2	12.3	109.0	17.8	99.4	6.6	114.9	19.1
AD-959856.1	120.8	10.0	112.1	6.2	142.0	30.4	86.1	4.2
AD-959906.1	87.2	12.3	112.3	15.7	119.5	3.6	123.0	9.0
AD-959936.1	95.8	11.2	112.6	17.9	108.7	12.1	133.5	20.9
AD-959896.1	86.2	10.8	114.9	9.7	104.9	12.0	111.6	13.5
AD-959893.1	110.4	6.1	115.5	23.1	102.0	14.7	112.9	9.6
AD-959892.1	97.9	20.0	118.4	10.8	97.9	9.2	114.0	14.5
AD-959894.1	118.2	10.8	132.2	25.9	103.5	3.9	134.0	17.1
AD-959750.1	150.2	3.6	136.5	12.9	113.4	7.3	135.5	21.5
AD-959898.1	129.1	11.8	139.1	29.1	125.4	12.1	137.4	13.5

	50 nM Dose		10 nM Dose		1.0 nM Dose		0.1 nM Dose	
Duplex	Avg % APOC3 mRNA Remaining	SD	Avg % APOC3 mRNA Remaining	SD	Avg % APOC3 mRNA Remaining	SD	Avg % APOC3 mRNA Remaining	SD
AD-959895.1	107.1	12.9	158.5	27.8	116.8	12.8	133.9	13.1

Table 7. APOC3 Single Dose Screens in Hep3B cells

	50 nM Dose		10 nM Dose		1.0 nM Dose		0.1 nM Dose	
Duplex	Avg % APOC3 mRNA Remaining	SD	Avg % APOC3 mRNA Remaining	SD	Avg % APOC3 mRNA Remaining	SD	Avg % APOC3 mRNA Remaining	SD
AD-960293.1	5.3	1.1	4.4	0.9	12.7	3.6	49.3	8.8
AD-960288.1	7.1	1.1	4.5	0.3	16.4	2.8	60.9	3.2
AD-960445.1	7.2	2.3	4.6	0.9	24.9	4.1	67.0	11.2
AD-960292.1	8.4	2.7	6.1	1.8	35.0	3.6	89.3	13.2
AD-960475.1	9.5	1.8	6.2	1.5	20.0	7.7	79.7	7.4
AD-960442.1	12.1	1.5	6.5	1.7	21.5	1.3	79.3	18.3
AD-960470.1	11.8	1.9	7.1	0.6	40.0	10.6	78.1	5.5
AD-960436.1	11.3	3.2	7.9	2.0	31.6	3.0	86.2	7.7
AD-960446.1	8.3	1.8	8.4	1.6	43.0	10.6	77.2	4.3
AD-960474.1	11.6	1.8	8.4	0.9	39.4	5.9	103.2	18.7
AD-960294.1	14.0	1.7	8.5	1.6	32.8	9.1	74.7	5.8
AD-960471.1	12.2	1.5	8.5	0.8	28.8	7.5	81.1	16.8
AD-960314.1	11.0	0.4	9.3	1.1	41.2	9.8	79.2	4.3
AD-960443.1	17.7	6.1	9.9	2.6	33.4	5.5	95.7	15.2
AD-960282.1	12.2	1.9	10.0	2.4	51.8	13.2	106.4	30.6
AD-960283.1	13.2	0.9	10.0	1.3	50.3	6.3	101.6	8.4
AD-80794.7	9.5	1.4	10.8	2.5	34.9	9.1	78.4	7.5
AD-960295.1	25.0	4.9	11.4	4.0	43.9	2.4	82.2	10.5
AD-960478.1	15.1	1.9	11.8	3.4	17.7	2.1	62.6	9.7
AD-960289.1	14.9	0.7	11.8	1.4	44.7	4.4	97.2	16.6
AD-960481.1	17.1	2.8	12.8	2.3	25.9	6.9	56.2	2.0
AD-960318.1	21.0	5.6	13.3	2.4	43.2	2.3	83.6	11.4
AD-960297.1	21.5	2.5	14.1	1.8	49.0	5.0	103.4	9.0
AD-960477.1	13.6	5.0	14.1	3.1	29.8	6.1	89.6	17.5
AD-960317.1	20.9	5.7	14.3	3.9	48.5	19.9	101.0	28.8
AD-960476.1	14.2	1.5	16.3	1.0	41.1	7.5	92.0	4.5
AD-960241.1	15.6	2.3	17.1	1.4	68.4	8.4	103.7	27.4
AD-960480.1	20.1	1.0	18.0	2.5	62.0	10.9	91.8	12.4
AD-960482.1	25.5	6.0	19.8	2.8	37.2	3.5	60.8	11.6
AD-80793.7	16.6	3.1	20.2	3.5	43.8	5.9	64.1	5.9
AD-960107.1	30.1	1.1	20.6	3.8	61.2	8.7	95.4	14.3
AD-960308.1	31.9	3.3	23.3	2.2	48.0	5.8	90.2	4.9
AD-960121.1	25.3	4.5	24.7	4.5	65.9	7.8	87.0	12.5

	50 nM Dose		10 nM Dose		1.0 nM Dose		0.1 nM Dose	
Duplex	Avg % APOC3 mRNA Remaining	SD	Avg % APOC3 mRNA Remaining	SD	Avg % APOC3 mRNA Remaining	SD	Avg % APOC3 mRNA Remaining	SD
AD-960287.1	37.5	6.9	24.8	10.3	90.3	22.4	82.9	5.2
AD-960473.1	22.4	5.3	25.1	3.4	98.0	19.4	104.8	24.8
AD-960479.1	28.3	4.1	25.4	8.2	72.6	18.2	90.5	8.2
AD-960278.1	22.8	5.6	25.6	5.4	71.9	8.8	105.8	15.8
AD-960113.1	26.5	4.8	25.7	7.0	59.0	5.7	114.0	17.3
AD-960472.1	38.2	8.0	26.5	8.2	56.0	8.7	107.3	28.4
AD-960444.1	26.5	4.8	27.3	3.0	72.4	12.5	127.2	16.8
AD-960303.1	30.6	4.3	28.4	6.1	82.0	15.0	104.9	20.2
AD-960438.1	31.3	4.4	28.6	6.0	59.2	6.1	90.3	14.4
AD-960290.1	29.8	5.6	30.3	3.9	80.8	4.3	107.1	12.4
AD-960304.1	32.6	7.7	30.4	3.0	86.3	9.9	100.4	15.6
AD-960388.1	29.6	2.0	30.7	2.2	71.8	11.7	102.8	27.2
AD-960233.1	28.4	4.7	31.5	5.7	96.7	11.8	99.0	17.2
AD-960234.1	46.3	7.2	33.1	8.5	93.3	10.2	97.3	14.5
AD-960114.1	15.9	2.5	33.2	4.3	58.9	4.5	93.9	11.3
AD-960296.1	49.5	5.3	34.5	9.2	70.8	7.0	93.6	8.3
AD-960431.1	40.7	8.8	35.1	1.1	87.2	3.7	108.8	8.6
AD-960316.1	35.5	2.6	37.2	8.2	78.9	14.8	93.4	15.1
AD-960307.1	25.4	1.3	37.4	4.6	90.9	7.7	99.5	10.8
AD-960120.1	21.4	1.7	37.8	5.6	86.5	12.6	107.1	16.1
AD-960238.1	20.5	2.9	37.9	9.9	79.7	22.5	128.8	11.2
AD-960301.1	35.6	6.4	38.2	7.4	75.6	9.0	101.6	21.1
AD-960235.1	32.1	6.1	39.4	12.7	107.9	8.9	167.9	37.6
AD-960123.1	29.3	3.4	42.8	3.8	85.3	7.9	101.2	19.9
AD-960300.1	24.4	3.1	45.3	13.1	77.9	12.3	101.2	15.3
AD-960285.1	64.5	4.8	46.0	6.9	86.7	12.6	98.6	9.4
AD-960469.1	43.0	6.1	46.3	6.8	72.4	12.5	96.4	20.8
AD-960387.1	40.5	2.6	46.6	16.0	94.0	8.6	99.9	6.8
AD-960384.1	42.8	2.9	47.1	2.9	103.6	9.9	103.2	13.1
AD-960109.1	41.1	5.7	48.6	6.8	66.1	7.0	81.6	10.0
AD-960112.1	44.6	2.4	48.8	5.3	69.0	17.5	103.7	12.3
AD-960386.1	59.3	4.9	49.2	10.1	100.5	20.4	106.1	13.2
AD-960302.1	69.2	12.5	49.4	10.1	82.4	14.5	106.2	14.6
AD-960118.1	44.1	5.1	49.7	10.8	98.1	23.5	136.2	29.0
AD-960111.1	43.3	6.9	50.0	4.5	93.6	9.3	107.0	20.0
AD-960299.1	44.7	5.2	51.2	4.0	105.0	11.8	114.8	7.7
AD-960115.1	42.3	6.0	52.1	2.6	82.4	12.1	85.4	15.0
AD-960439.1	51.3	16.3	52.8	1.8	92.2	13.9	98.5	12.5
AD-960441.1	74.3	9.3	53.2	5.7	98.1	21.9	102.2	20.2
AD-960232.1	43.3	6.8	54.7	4.6	105.5	8.5	109.6	5.2
AD-960276.1	39.7	10.0	54.9	21.4	101.5	29.8	107.1	22.3

	50 nM Dose		10 nM Dose		1.0 nM Dose		0.1 nM Dose	
Duplex	Avg % APOC3 mRNA Remaining	SD	Avg % APOC3 mRNA Remaining	SD	Avg % APOC3 mRNA Remaining	SD	Avg % APOC3 mRNA Remaining	SD
AD-960435.1	64.2	1.1	57.2	4.9	85.5	12.3	109.9	13.3
AD-960306.1	57.8	4.6	57.8	3.7	74.1	15.0	91.4	10.5
AD-960172.1	39.5	1.4	57.8	3.6	117.6	27.7	115.2	36.2
AD-960385.1	89.3	7.4	59.2	19.5	124.6	37.9	107.8	16.4
AD-960110.1	36.5	2.7	62.2	15.6	64.7	6.4	82.5	12.4
AD-960116.1	26.8	3.5	62.4	10.8	78.8	10.9	81.0	12.9
AD-960434.1	64.8	7.8	63.1	19.2	79.0	6.8	111.8	14.9
AD-960430.1	75.9	11.5	67.0	8.7	111.9	8.6	103.9	4.9
AD-960305.1	54.3	7.1	68.7	18.0	83.3	9.3	106.2	24.0
AD-960279.1	97.3	25.7	69.3	1.5	138.6	43.7	108.2	17.4
AD-960298.1	82.1	2.0	72.0	18.4	81.0	18.2	97.0	22.4
AD-960284.1	91.4	20.3	74.0	11.1	103.5	15.0	115.2	20.0
AD-960313.1	88.5	3.6	74.6	18.9	116.9	17.3	96.1	8.5
AD-960432.1	77.1	6.5	76.4	8.3	94.7	20.2	103.7	10.5
AD-960124.1	62.0	6.5	76.7	9.3	110.4	22.7	115.3	9.8
AD-960119.1	78.8	6.0	77.7	6.6	106.5	24.1	140.7	8.0
AD-960437.1	101.3	8.8	77.7	11.6	98.4	23.9	116.1	21.1
AD-960315.1	84.1	10.8	79.6	8.0	107.5	13.0	103.5	4.3
AD-960117.1	82.4	28.4	81.8	8.8	126.7	10.4	118.9	17.6
AD-960311.1	82.6	12.2	82.3	8.7	138.1	20.4	112.0	24.6
AD-960272.1	90.8	17.5	84.1	4.2	114.7	13.2	90.1	4.8
AD-960414.1	105.6	23.4	84.5	4.9	117.2	13.8	113.6	24.8
AD-960240.1	81.0	9.2	85.6	14.5	98.0	5.1	95.5	27.7
AD-960286.1	87.4	6.6	85.8	11.8	122.0	17.0	101.7	2.6
AD-960281.1	119.9	27.1	86.6	23.2	120.5	17.4	112.4	6.8
AD-960277.1	126.8	14.6	94.8	21.3	166.0	68.5	99.9	13.6
AD-960274.1	120.6	27.3	95.6	17.5	128.4	17.1	123.9	27.1
AD-960108.1	79.8	6.1	95.8	17.5	103.7	9.4	96.7	11.4
AD-960239.1	106.5	19.7	95.9	13.8	112.7	16.0	109.3	10.7
AD-960122.1	100.1	10.3	96.3	3.1	132.0	3.7	117.6	8.3
AD-960291.1	111.5	17.5	96.3	18.2	99.9	18.0	125.3	23.6
AD-960125.1	134.2	17.5	98.0	12.3	122.9	17.1	100.1	12.4
AD-960231.1	95.9	17.6	99.4	23.7	99.8	27.2	99.9	23.1
AD-960275.1	69.0	6.8	99.7	7.9	93.7	14.8	137.6	22.2
AD-960173.1	81.3	13.7	99.8	30.0	96.3	19.1	104.6	28.9
AD-960271.1	94.3	8.3	100.1	5.2	106.7	13.0	101.9	14.3
AD-960433.1	165.5	10.3	101.1	10.9	145.3	26.4	101.8	9.0
AD-960267.1	114.1	6.1	101.4	8.4	105.4	23.3	112.1	4.1
AD-960236.1	95.8	5.7	101.8	10.5	106.6	25.8	110.3	13.0
AD-960310.1	112.9	9.5	103.5	13.1	106.6	16.8	112.8	19.0
AD-960312.1	106.2	3.8	103.8	17.4	147.1	31.1	107.2	8.8

	50 nM Dose		10 nM Dose		1.0 nM Dose		0.1 nM Dose	
Duplex	Avg % APOC3 mRNA Remaining	SD	Avg % APOC3 mRNA Remaining	SD	Avg % APOC3 mRNA Remaining	SD	Avg % APOC3 mRNA Remaining	SD
AD-960309.1	91.5	6.4	107.7	14.0	104.1	14.2	100.7	23.9
AD-960440.1	106.8	12.6	110.3	15.5	89.7	23.6	108.7	17.4
AD-960237.1	129.8	8.4	113.2	15.2	101.1	18.6	127.7	5.6
AD-960268.1	100.0	8.7	114.3	5.7	97.1	5.1	89.3	16.4
AD-960269.1	134.7	14.3	118.2	10.1	107.5	21.8	131.1	22.3
AD-960280.1	83.9	4.5	121.9	23.0	116.0	20.5	118.5	41.7
AD-960270.1	122.8	11.5	122.1	13.0	102.7	15.9	114.3	16.2
AD-960273.1	149.2	4.7	142.6	33.3	107.4	10.5	145.7	16.6

Example 3. *In vivo* screening of dsRNA Duplexes in Mice

Duplexes of interest, identified from the above *in vitro* studies, were evaluated *in vivo*. In particular, at pre-dose day -14 wild-type mice (C57BL/6) were transduced by retroorbital administration of 2×10^{10} viral particles of an adeno-associated virus 8 (AAV8) vector encoding human APOC3. In particular, mice were administered an AAV8 encoding the human APOC3 mRNA, referred to as AAV8-TBG-PI-APOC3.

At day 0, groups of three mice were subcutaneously administered a single 3 mg/kg dose of the agents of interest or PBS control. Table 8 provides the treatment groups and Table 9 provides the modified nucleotide sequences of the sense and antisense strands of the duplexes of interest. At day 7 or day 14 post-dose animals were sacrificed, liver samples were collected and snap-frozen in liquid nitrogen. Liver mRNA was extracted and analyzed by the RT-QPCR method.

Human APOC3 mRNA levels were compared to a housekeeping gene, GAPDH. The values were then normalized to the average of PBS vehicle control group. The data were expressed as percent of baseline value, and presented as mean plus standard deviation. The results, listed in Table 10 and shown in Figure 1, demonstrate that the exemplary duplex agents tested effectively reduce the level of the human APOC3 messenger RNA *in vivo*.

Table 8. Treatment Groups

Group #	Animal #	Treatment	Dose	Timepoint
1	1	PBS	n/a	D0, D7, D14
	2			
	3			
2	4	Naïve	n/a	
	5			
	6			

3	7	AD-959917.1	3mpk
	8		
	9		
4	10	AD-960064.1	
	11		
	12		
5	13	AD-960293.1	
	14		
	15		
6	16	AD-960288.1	
	17		
	18		
7	19	AD-960445.1	
	20		
	21		
8	22	AD-960292.1	
	23		
	24		
9	25	AD-960475.1	
	26		
	27		
10	28	AD-960442.1	
	29		
	30		
11	31	AD-960470.1	
	32		
	33		
12	34	AD-960446.1	
	35		
	36		
13	37	AD-960436.1	
	38		
	39		
14	40	AD-960443.1	
	41		
	42		
15	43	AD-960063.1	
	44		
	45		
16	46	AD-960031.1	
	47		
	48		
17	49	AD-959910.1	

	50		
	51		
18	52	AD-960096.1	
	53		
	54		
19	55	AD-959918.1	
	56		
	57		
20	58	AD-80794.7	
	59		
	60		

Table 9. Duplexes of Interest

Duplex ID	Range in NM-000040.3
AD-959917.1	243-265
AD-960064.1	433-455
AD-960031.1	431-453
AD-960063.1	431-453
AD-960293.1	243-265
AD-960288.1	238-260
AD-960445.1	435-457
AD-960292.1	242-264
AD-960475.1	504-526
AD-960442.1	432-454
AD-960470.1	499-521
AD-960436.1	426-448
AD-960446.1	436-458
AD-960474.1	503-525
AD-960294.1	244-266
AD-960443.1	433-455
AD-80794.7	430-450
AD-959910.1	235-257

Table 10.

	D14			
	Liver RTqPCR		ELISA	
Duplex	% Message Remaining	SD	Avg	SEM
PBS	100.56	10.81	173.05	34.36
Naïve	105.57	1.84	125.40	46.86
AD-959917.1	38.42	16.51	63.57	13.65
AD-960064.1	16.01	2.36	36.97	15.66
AD-960293.1	60.82	17.10	119.00	12.04

AD-960288.1	75.24	14.48	99.64	12.46
AD-960445.1	19.60	7.46	61.84	1.26
AD-960292.1	91.68	22.51	78.59	3.24
AD-960475.1	61.18	20.12	73.65	14.56
AD-960442.1	55.63	17.52	91.45	8.10
AD-960470.1	31.48	9.94	87.10	13.53
AD-960446.1	34.44	5.34	62.84	11.40
AD-960436.1	35.50	9.98	68.61	17.47
AD-960443.1	60.00	3.61	88.72	8.31
AD-960063.1	14.64	7.30	42.19	2.84
AD-960031.1	11.45	5.18	20.57	5.87
AD-959910.1	67.13	13.87	59.45	0.75
AD-960096.1	22.96	8.58	13.15	3.40
AD-959918.1	76.69	7.97	51.37	3.33
AD-80794.7	23.00	15.59	17.38	3.01

Additional duplexes of interest, identified from the above *in vitro* studies, were also evaluated *in vivo*. In particular, at pre-dose day -14 wild-type mice (C57BL/6) were transduced by retroorbital administration of 2×10^{10} viral particles of an adeno-associated virus 8 (AAV8) vector encoding human APOC3. In particular, mice were administered an AAV8 encoding the human APOC3 mRNA, referred to as AAV8-TBG-PI-APOC3.

At day 0, groups of three mice were subcutaneously administered a single 3 mg/kg dose of the agents of interest or PBS control. Table 11 provides the treatment groups and Table 12 provides the modified nucleotide sequences of the sense and antisense strands of the duplexes of interest. At day 7 or day 14 post-dose animals were sacrificed, liver samples were collected and snap-frozen in liquid nitrogen. Liver mRNA was extracted and analyzed by the RT-QPCR method.

Human APOC3 mRNA levels were compared to a housekeeping gene, GAPDH. The values were then normalized to the average of PBS vehicle control group. The data were expressed as percent of baseline value, and presented as mean plus standard deviation. The results, listed in Table 13 and shown in Figure 2, demonstrate that the exemplary duplex agents tested effectively reduce the level of the human APOC3 messenger RNA *in vivo*.

Table 11. Treatment Groups

Group #	Animal #	Treatment	Dose	Timepoint
1	1	PBS	n/a	D0, D7, D14
	2			
	3			
2	4	Naïve	n/a	
	5			
	6			

3	7	AD-80794	3mpk	
	8			
	9			
4	10	AD-959907		
	11			
	12			
5	13	AD-959914		
	14			
	15			
6	16	AD-959916		
	17			
	18			
7	19	AD-959932		
	20			
	21			
8	22	AD-959941		
	23			
	24			
9	25	AD-960030		
	26			
	27			
10	28	AD-960062		
	29			
	30			
11	31	AD-960064		
	32			
	33			
12	34	AD-960065		
	35			
	36			
13	37	AD-960066		
	38			
	39			
14	40	AD-960294		
	41			
	42			
15	43	AD-960314		
	44			
	45			
16	46	AD-960471		
	47			
	48			
17	49	AD-960474		

	50			
	51			
18	52	AD-960478		
	53			
	54			
19	55	AD-960481		
	56			
	57			

Table 12. Additional Duplexes of Interest

Duplex ID	Range in NM-000040.3
AD-80794.8	430-450
AD-959907.2	232-254
AD-959914.2	239-261
AD-959916.2	242-264
AD-959932.2	258-280
AD-960314.2	264-286
AD-959941.2	268-290
AD-960030.2	429-451
AD-960062.2	430-452
AD-960064.2	433-455
AD-960065.2	434-456
AD-960066.2	435-457
AD-960294.2	244-266
AD-960471.2	500-522
AD-960474.2	503-525
AD-960478.2	507-529
AD-960481.2	510-532

Table 13.

Duplex	% Message Remaining	SD
PBS	102.94	34.16
Naïve	97.12	15.32
AD-80794	15.17	8.74
AD-959907	49.60	35.60
AD-959914	75.84	20.55
AD-959916	51.81	27.99
AD-959932	62.36	13.32
AD-959941	55.60	17.44
AD-960030	10.26	2.93
AD-960062	24.91	9.81
AD-960064	20.04	8.94

AD-960065	38.62	18.89
AD-960066	20.93	5.78
AD-960294	73.30	24.03
AD-960314	48.32	31.10
AD-960471	39.24	28.51
AD-960474	30.81	15.15
AD-960478	47.27	7.36
AD-960481	37.22	9.27

Example 4. Structure-Activity Relationship Analyses

Based on the *in vitro* analyses in Example 2 and the *in vivo* analyses in Example 4, structure-active relationship (SAR) analyses were performed. In particular, additional duplexes were designed, synthesized, and assayed *in vitro* and *in vivo*. The additional agents were designed to target within nucleotides 429-455 or nucleotides 504-532 of NM_000040.3.

siRNAs were synthesized and annealed using routine methods known in the art and described above.

Detailed lists of the unmodified APOC3 sense and antisense strand nucleotide sequences are shown in Table 14. Detailed lists of the modified apolipoprotein C3 sense and antisense strand nucleotide sequences are shown in Table 15.

For free uptake, experiments were performed by adding 2.5 μ L of siRNA duplexes in PBS per well into a 96 well plate. Complete growth media (47.5 μ L) containing about 1.5×10^4 Hep3B cells was then added to the siRNA. Cells were incubated for 48 hours prior to RNA purification and RT-qPCR, as described above. Single dose experiments were performed at 500nM, 100 nM, 10 nM, and 1nM final duplex concentration.

For transfections, Hep3b cells (ATCC, Manassas, VA) were grown to near confluence at 37°C in an atmosphere of 5% CO₂ in Eagle's Minimum Essential Medium (Gibco) supplemented with 10% FBS (ATCC) before being released from the plate by trypsinization. Transfection was carried out by adding 7.5 μ L of Opti-MEM plus 0.1 μ L of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) to 2.5 μ L of each siRNA duplex to an individual well in a 384-well plate. The mixture was then incubated at room temperature for 15 minutes. Forty μ L of complete growth media without antibiotic containing $\sim 1.5 \times 10^4$ Hep3B cells were then added to the siRNA mixture. Cells were incubated for 24 hours prior to RNA purification. Single dose experiments were performed at 50, nM, 10 nM, 1 nM, and 0.1 nM final duplex concentration.

Total RNA isolation was performed using DYNABEADS. Briefly, cells were lysed in 10 μ L of Lysis/Binding Buffer containing 3 μ L of beads per well and mixed for 10 minutes on an electrostatic shaker. The washing steps were automated on a Biotek EL406, using a magnetic plate support. Beads were washed (in 3 μ L) once in Buffer A, once in Buffer B, and twice in Buffer E, with aspiration steps in between. Following a final aspiration, complete 12 μ L RT mixture was added to each well, as described below.

For cDNA synthesis, a master mix of 1.5µl 10X Buffer, 0.6µl 10X dNTPs, 1.5µl Random primers, 0.75µl Reverse Transcriptase, 0.75µl RNase inhibitor and 9.9µl of H₂O per reaction were added per well. Plates were sealed, agitated for 10 minutes on an electrostatic shaker, and then incubated at 37 degrees C for 2 hours. Following this, the plates were agitated at 80 degrees C for 8
5 minutes.

RT-qPCR was performed as described above and relative fold change was calculated as described above.

The results of the free uptake experiments of the dsRNA agents listed in Tables 14 and 15 are shown in Table 16 and the results of the transfection assays of the dsRNA agents listed in Tables 14
10 and 15 in Hep3B cells are shown in Table 17.

Table 14. Unmodified Sense and Antisense Strand Sequences of Apolipoprotein C3 dsRNA Agents

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000040.3	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000040.3
AD-80794.10	CUUAAAAGGGACAGUAUUCUA	13	434-452	UAGAAUACUGUCCCCUUUUAAGCA	58	432-452
AD-1143240.1	CUUAAAAGGGACAGUAUUCUA	13	434-452	UAGAAUACUGUCCCCUUUUAAGCA	58	432-452
AD-1143241.1	CUUAAAAGGGACAGUAUUCUA	13	434-452	UAGAAUACUGUCCCCUUUUAAGCA	58	432-452
AD-1143242.1	CUUAAAAGGGACAGUAUUCUA	13	434-452	UAGAAUACUGUCCCCUUUUAAGCC	14	432-452
AD-1143243.1	CUUAAAAGGGACAGUAUUCUA	13	434-452	UAGAAUACUGUCCCCUUUUAAGCC	14	432-452
AD-1143244.1	UAAAAGGGACAGUAUUCUA	299	434-452	UAGAAUACUGUCCCCUUUUAAG	300	432-452
AD-1143245.1	UAAAAGGGACAGUAUUCUA	299	434-452	UAGAAUACUGUCCCCUUUUAAG	300	432-452
AD-1143246.1	CUUAAAAGGGACAGUAUUCUA	13	432-452	UAGAAUACUGUCCCCUUUUAAGCC	14	430-452
AD-1143247.1	CUUAAAAGGGACAGUAUUCUA	13	432-452	UAGAAUACUGUCCCCUUUUAAGCC	14	430-452
AD-1143248.1	CUUAAAAGGGACAGUAUUCUA	13	432-452	UAGAAUACUGUCCCCUUUUAAGCC	14	430-452
AD-1143249.1	CUUAAAAGGGACAGUAUUCUA	13	432-452	UAGAAUACUGUCCCCUUUUAAGCC	14	430-452
AD-960030.3	GCUUAAAAGGGACAGUAUUCU	56	431-451	AGAAUACUGUCCCCUUUUAAGCAA	57	429-451
AD-1143250.1	GCUUAAAAGGGACAGUAUUCU	56	431-451	AGAAUACUGUCCCCUUUUAAGCAA	57	429-451
AD-1143251.1	GCUUAAAAGGGACAGUAUUCU	56	431-451	AGAAUACUGUCCCCUUUUAAGCAA	57	429-451
AD-1143252.1	GCUUAAAAGGGACAGUAUUCU	56	431-451	AGAATACUGUCCCCUUUUAAGCAA	301	429-451
AD-1143253.1	GCUUAAAAGGGACAGUAUUCU	56	431-451	AGAAUACUGUCCCCUUUUAAGCGC	302	429-451
AD-1143254.1	GCUUAAAAGGGACAGUAUUCU	56	431-451	AGAATACUGUCCCCUUUUAAGCGC	303	429-451
AD-1143255.1	UUAAAAGGGACAGUAUUCU	304	433-451	AGAAUACUGUCCCCUUUUAAGC	305	431-451
AD-1143256.1	UUAAAAGGGACAGUAUUCU	304	433-451	AGAATACUGUCCCCUUUUAAGC	306	431-451
AD-1143257.1	GCUUAAAAGGGACAGUAUUCU	56	431-451	AGAAUACUGUCCCCUUUUAAGCGC	302	429-451
AD-1143258.1	GCUUAAAAGGGACAGUAUUCU	56	431-451	AGAATACUGUCCCCUUUUAAGCGC	303	429-451
AD-1143259.1	GCUUAAAAGGGACAGUAUUCU	56	431-451	AGAAUACUGUCCCCUUUUAAGCGC	302	429-451
AD-1143260.1	GCUUAAAAGGGACAGUAUUCU	56	431-451	AGAATACUGUCCCCUUUUAAGCGC	303	429-451
AD-960031.3	UAAAAGGGACAGUAUUCUAU	36	434-454	AUGAGAAUACUGUCCCCUUUUAAG	37	432-454

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000040.3	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000040.3
AD-1143261.1	UAAAAAGGGACAGUAUUUUCAU	307	434-454	AUGAAAAUACUGUCCCUUUUAAAG	308	432-454
AD-1143262.1	UAAAAAGGGACAGUAUUUUCAU	36	434-454	AUGAGAAUACUGUCCCUUUUAAAG	37	432-454
AD-1143263.1	UAAAAAGGGACAGUAUUUUCAU	36	434-454	AUGAGAAUACUGUCCCUUUUAAAG	37	432-454
AD-1143264.1	UAAAAAGGGACAGUAUUUUCAU	36	434-454	AUGAGAAUACUGUCCCUUUUAAAG	309	432-454
AD-1143265.1	UAAAAAGGGACAGUAUUUUCAU	36	434-454	AUGAGAAUACUGUCCCUUUUAAAG	309	432-454
AD-1143266.1	AAAGGGACAGUAUUUUCAU	310	436-454	AUGAGAAUACUGUCCCUUUUAAAG	311	434-454
AD-1143267.1	AAAGGGACAGUAUUUUCAU	310	436-454	AUGAGAAUACUGUCCCUUUUAAAG	311	434-454
AD-1143268.1	UAAAAAGGGACAGUAUUUUCAU	36	434-454	AUGAGAAUACUGUCCCUUUUAAAG	309	432-454
AD-1143269.1	UAAAAAGGGACAGUAUUUUCAU	312	434-454	AUGAGAAUACUGUCCCUUUUAAAG	313	432-454
AD-1143270.1	UAAAAAGGGACAGUAUUUUCAU	36	434-454	AUGAGAAUACUGUCCCUUUUAAAG	309	432-454
AD-1143271.1	UAAAAAGGGACAGUAUUUUCAU	314	434-454	AUGAGAAUACUGUCCCUUUUAAAG	309	432-454
AD-1143272.1	UAAAAAGGGACAGUAUUUUCAU	314	434-454	AUGAGAAUACUGUCCCUUUUAAAG	309	432-454
AD-1143273.1	UAAAAAGGGACAGUAUUUUCAU	36	434-454	AUGAGAAUACUGUCCCUUUUAAAG	37	432-454
AD-1143274.1	UAAAAAGGGACAGUAUUUUCAU	314	434-454	AUGAGAAUACUGUCCCUUUUAAAG	37	432-454
AD-960062.3	CUUAAAAGGGACAGUAUUUUCU	48	432-452	AAGAAUACUGUCCCUUUUAAAGCA	49	430-452
AD-1143275.1	CUUAAAAGGGACAGUAUUUUCU	48	432-452	AAGAAUACUGUCCCUUUUAAAGCA	49	430-452
AD-1143276.1	CUUAAAAGGGACAGUAUUUUCU	48	432-452	AAGAAUACUGUCCCUUUUAAAGCA	49	430-452
AD-1143277.1	CUUAAAAGGGACAGUAUUUUCU	48	432-452	AAGAAUACUGUCCCUUUUAAAGCC	315	430-452
AD-1143278.1	CUUAAAAGGGACAGUAUUUUCU	48	432-452	AAGAAUACUGUCCCUUUUAAAGCC	315	430-452
AD-1143279.1	UAAAAAGGGACAGUAUUUUCU	316	434-452	AAGAAUACUGUCCCUUUUAAAG	317	432-452
AD-1143280.1	UAAAAAGGGACAGUAUUUUCU	316	434-452	AAGAAUACUGUCCCUUUUAAAG	317	432-452
AD-1143281.1	CUUAAAAGGGACAGUAUUUUCU	48	432-452	AAGAAUACUGUCCCUUUUAAAGCC	315	430-452
AD-1143282.1	CUUAAAAGGGACAGUAUUUUCU	48	432-452	AAGAAUACUGUCCCUUUUAAAGCC	315	430-452
AD-1143283.1	CUUAAAAGGGACAGUAUUUUCU	48	432-452	AAGAAUACUGUCCCUUUUAAAGCC	315	430-452
AD-1143284.1	CUUAAAAGGGACAGUAUUUUCU	48	432-452	AAGAAUACUGUCCCUUUUAAAGCC	315	430-452

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000040.3	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000040.3
AD-960064.3	AAAAGGGACAGUAUUCUCAGU	30	435-455	ACUGAGAAUACUGUCCCCUUUUA	31	433-455
AD-1143285.1	AAAAGGGACAGUAUUCUCAGU	30	435-455	ACUGAGAAUACUGUCCCCUUUUA	31	433-455
AD-1143286.1	AAAAGGGACAGUAUUCUCAGU	30	435-455	ACUGAGAAUACUGUCCCCUUUUA	31	433-455
AD-1143287.1	AAAAGGGACAGUAUUCUCAGU	30	435-455	ACUGAGAAUACUGUCCCCUUUUGC	318	433-455
AD-1143288.1	AAAAGGGACAGUAUUCUCAGU	30	435-455	ACUGAGAAUACUGUCCCCUUUUC	319	433-455
AD-1143289.1	AAGGGACAGUAUUCUCAGU	320	437-455	ACUGAGAAUACUGUCCCCUUU	321	435-455
AD-1143290.1	AAAAGGGACAGUAUUCUCAGU	30	435-455	ACUGAGAAUACUGUCCCCUUUUGC	318	433-455
AD-1143291.1	AAAAGGGACAGUAUUCUCAGU	30	435-455	ACUGAGAAUACUGUCCCCUUUUC	319	433-455
AD-1143292.1	AAAAGGGACAGUAUUCUCAGU	322	435-455	ACUGAGAAUACUGUCCCCUUUUGC	323	433-455
AD-1143293.1	AAAAGGGACAGUAUUCUCAGU	30	435-455	ACUGAGAAUACUGUCCCCUUUUGC	318	433-455
AD-1143294.1	AAAAGGGACAGUAUUCUCAGU	30	435-455	ACUGAGAAUACUGUCCCCUUUUC	319	433-455
AD-1143295.1	AAAAGGGACAGUAUUCUCAGU	30	435-455	ACUGAGAAUACUGUCCCCUUUUGC	318	433-455
AD-1143296.1	AAAAGGGACAGUAUUCUCAGU	30	435-455	ACUGAGAAUACUGUCCCCUUUUC	319	433-455
AD-960096.3	CCAAUAAAGCUGGACAAGAAU	28	506-526	AUUCUUGUCCAGCUUUUAUUGGGA	29	504-526
AD-1143297.1	CCAAUAAAGCUGGACAAGAAU	324	506-526	AUUCUUAUCCAGCUUUUAUUGGGA	325	504-526
AD-1143298.1	CCAAUAAAGCUGGACAAGAAU	28	506-526	AUUCTUGUCCAGCUUUUAUUGGGA	326	504-526
AD-1143299.1	CCAAUAAAGCUGGACAAGAAU	28	506-526	AUUCTUGUCCAGCUUUUAUUGGGA	327	504-526
AD-1143300.1	AAUAAAGCUGGACAAGAAU	328	508-526	AUUCTUGUCCAGCUUUUAUUGG	329	506-526
AD-1143301.1	AAUAAAGCUGGACAAGAAU	328	508-526	AUUCTUGUCCAGCUUUUAUUC	330	506-526
AD-1143302.1	CCAAUAAAGCUGGACAAGAAU	28	506-526	AUUCTUGUCCAGCUUUUAUUGGGA	327	504-526
AD-1143303.1	CCAAUAAAGCUGGACAAGAAU	28	506-526	AUUCTUGUCCAGCUUUUAUUGG	329	506-526
AD-1143304.1	CCAAUAAAGCUGGACAAGAAU	28	506-526	AUUCTUGUCCAGCUUUUAUUGGGA	327	504-526
AD-1143305.1	CCAAUAAAGCUGGACAAGAAU	28	506-526	AUUCTUGUCCAGCUUUUAUUGG	329	506-526
AD-1143306.1	CCAAUAAAGCUGGACAAGAAU	28	506-526	AUUCUUGUCCAGCUUUUAUUGGGA	29	504-526
AD-1143307.1	CCAAUAAAGCUGGACAAGAAU	28	506-526	AUUCUUGUCCAGCUUUUAUUGGGA	29	504-526

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000040.3	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000040.3
AD-960481.3	AAGCUGGACAAAGAGCUGCUU	99	512-532	AAGCAGCUUCUUGUCCAGCUUUA	100	510-532
AD-1143308.1	AAGCUGGACAAAGAGCUGCUU	99	512-532	AAGCAGCUUCUUGUCCAGCUUUA	100	510-532
AD-1143309.1	AAGCUGGACAAAGAGCUGCUU	99	512-532	AAGCAGCUUCUUGUCCAGCUUUA	100	510-532
AD-1143310.1	AAGCUGGACAAAGAGCUGCUU	99	512-532	AAGCAGCUUCUUGUCCAGCUUUA	100	510-532
AD-1143311.1	AAGCUGGACAAAGAGCUGCUU	99	512-532	AAGCAGCUUCUUGUCCAGCUUUC	331	510-532
AD-1143312.1	AAGCUGGACAAAGAGCUGCUU	99	512-532	AAGCAGCUUCUUGUCCAGCUUUC	331	510-532
AD-1143313.1	AAGCUGGACAAAGAGCUGCUU	99	512-532	AAGCAGCUUCUUGUCCAGCUUCC	332	510-532
AD-1143314.1	AAGCUGGACAAAGAGCUGCUU	99	512-532	AAGCAGCUUCUUGUCCAGCUUCC	332	510-532
AD-1143315.1	GCUGGACAAAGAGCUGCUU	333	514-532	AAGCAGCUUCUUGUCCAGCUU	334	512-532
AD-1143316.1	GCUGGACAAAGAGCUGCUU	333	514-532	AAGCAGCUUCUUGUCCAGCUU	334	512-532
AD-1143317.1	AAGCUGGACAAAGAGCUGCUU	99	512-532	AAGCAGCUUCUUGUCCAGCUUCC	332	510-532
AD-1143318.1	AAGCUGGACAAAGAGCUGCUU	99	512-532	AAGCAGCUUCUUGUCCAGCUUCC	332	510-532
AD-1143319.1	AAGCUGGACAAAGAGCUGCUU	99	512-532	AAGCAGCUUCUUGUCCAGCUUCC	332	510-532
AD-1143320.1	AAGCUGGACAAAGAGCUGCUU	99	512-532	AAGCAGCUUCUUGUCCAGCUUCC	332	510-532
AD-1143321.1	AAGCUGGACAAAGAGCUACUU	335	512-532	AAGUAGCUUCUUGUCCAGCUUCC	336	510-532
AD-1143322.1	AAGCUGGACAAAGAGUUGCUU	337	512-532	AAGCAACUUUCUUGUCCAGCUUCC	338	510-532
AD-1183925	AAGCUGGACAAAGAGCUGCUU	99	512-532	AAGCAGCUUCUUGUCCAGCUUUC	331	510-532
AD-1183926	GCUGGACAAAGAGCUGCUU	333	514-532	AAGCAGCUUCUUGUCCAGCUU	334	512-532
AD-1019001	ACGGGACAGUAUUCUCAGUA	339	437-456	UCACUGAGAAUACUGUCCCGU	340	437-457

Table 15. Modified Sense and Antisense Strand Sequences of Apolipoprotein C3 dsRNA Agents

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-80794.10	csusuaaaAfgGfGfAfcaguanucuaL96	17	usAfskaaUfaCfUfuccCfuUfuaagscsa	392	CUUAAAAGGGACAGUAUUCUC	828
AD-1143240.1	csusuaaaAfgGfGfAfcaguanucuaL96	17	usAfskaaUfacuguccCfuUfuaagscsa	829	CUUAAAAGGGACAGUAUUCUC	828
AD-1143241.1	csusuaaaAfgGfGfAfcaguanucuaL96	17	usdAsgaaUfacuguccCfuUfuaagscsa	830	CUUAAAAGGGACAGUAUUCUC	828
AD-1143242.1	csusuaaaAfgGfGfAfcaguanucuaL96	17	usAfskaaUfacuguccCfuUfuaagscsc	831	CUUAAAAGGGACAGUAUUCUC	828
AD-1143243.1	csusuaaaAfgGfGfAfcaguanucuaL96	17	usdAsgadAudAcuguccCfuUfuaagscsc	16	CUUAAAAGGGACAGUAUUCUC	828
AD-1143244.1	usasaaAfgGfGfAfcaguanucuaL96	832	usAfskaaUfacuguccCfuUfuaasag	833	CUUAAAAGGGACAGUAUUCUC	828
AD-1143245.1	usasaaAfgGfGfAfcaguanucuaL96	832	usdAsgadAudAcuguccCfuUfuaasag	834	CUUAAAAGGGACAGUAUUCUC	828
AD-1143246.1	csusuaaaagdGgdAcaguanucuaL96	835	usAfskaaUfacuguccCfuUfuaagscsc	831	UGCuuAAAAAGGGACAGUAUUCUC	379
AD-1143247.1	csusuaaaagdGgdAcaguanucuaL96	835	usdAsgadAudAcuguccCfuUfuaagscsc	16	UGCuuAAAAAGGGACAGUAUUCUC	379
AD-1143248.1	csusuaaaagdGgdACfaguanucuaL96	836	usAfskaaUfacuguccCfuUfuaagscsc	831	UGCuuAAAAAGGGACAGUAUUCUC	379
AD-1143249.1	csusuaaaagdGgdACfaguanucuaL96	836	usdAsgadAudAcuguccCfuUfuaagscsc	16	UGCuuAAAAAGGGACAGUAUUCUC	379
AD-960030.3	gscsuuaaAfaGfGfGfAfcaguanucuaL96	389	asGfsaaUfUfGfuccCfuUfuaagscsa	390	UUGCuuAAAAAGGGACAGUAUUCU	391
AD-1143250.1	gscsuuaaAfaGfGfGfAfcaguanucuaL96	389	asGfsaaUfUfGfuccCfuUfuaagscsa	837	UUGCuuAAAAAGGGACAGUAUUCU	391
AD-1143251.1	gscsuuaaAfaGfGfGfAfcaguanucuaL96	389	asdGsaauAfcuguccCfuUfuaagscsa	838	UUGCuuAAAAAGGGACAGUAUUCU	391
AD-1143252.1	gscsuuaaAfaGfGfGfAfcaguanucuaL96	389	asdGsaadTadCuguccCfuUfuaagscsa	839	UUGCuuAAAAAGGGACAGUAUUCU	391
AD-1143253.1	gscsuuaaAfaGfGfGfAfcaguanucuaL96	389	asdGsaauAfcuguccCfuUfuaagscsc	840	UUGCuuAAAAAGGGACAGUAUUCU	391
AD-1143254.1	gscsuuaaAfaGfGfGfAfcaguanucuaL96	389	asdGsaadTadCuguccCfuUfuaagscsc	841	UUGCuuAAAAAGGGACAGUAUUCU	391

AD-1143255.1	ususaaAfaGfGfGfacaguanuculL96	842	asdGsaauAfcugueccUfuUfuaagsgsc	843	GCUUAAAAGGGACAGUAUUCU	844
AD-1143256.1	ususaaAfaGfGfGfacaguanuculL96	842	asdGsaadTadCugueccUfuUfuaagsgsc	845	GCUUAAAAGGGACAGUAUUCU	844
AD-1143257.1	gscsuuaaaadGgdGacaguanuculL96	846	asdGsaauAfcugueccUfuUfuaagsgsc	840	UUGCUUAAAAGGGACAGUAUUCU	391
AD-1143258.1	gscsuuaaaadGgdGacaguanuculL96	846	asdGsaadTadCugueccUfuUfuaagsgsc	841	UUGCUUAAAAGGGACAGUAUUCU	391
AD-1143259.1	gscsuuaaaadGgdGadCaguanuculL96	847	asdGsaauAfcugueccUfuUfuaagsgsc	840	UUGCUUAAAAGGGACAGUAUUCU	391
AD-1143260.1	gscsuuaaaadGgdGadCaguanuculL96	847	asdGsaadTadCugueccUfuUfuaagsgsc	841	UUGCUUAAAAGGGACAGUAUUCU	391
AD-960031.3	usasaagGfgAfCfAfguanucucalL96	359	asUfsgagAfaUfafeuguCfcCfuuuuasag	360	CUUAAAAGGGACAGUAUUCUCAG	361
AD-1143261.1	usasaagGfgAfCfAfguanucucalL96	848	asUfsgaaAfaUfafeuguCfcCfuuuuasag	849	CUUAAAAGGGACAGUAUUCUCAG	361
AD-1143262.1	usasaagGfgAfCfAfguanucucalL96	359	asUfsgadGa(Agn)uacuguCfcCfuuuuasag	850	CUUAAAAGGGACAGUAUUCUCAG	361
AD-1143263.1	usasaagGfgAfCfAfguanucucalL96	359	asUfsgadGa(A2p)uacuguCfcCfuuuuasag	851	CUUAAAAGGGACAGUAUUCUCAG	361
AD-1143264.1	usasaagGfgAfCfAfguanucucalL96	359	asUfsgadGa(Agn)uacuguCfcCfuuuuasag	852	CUUAAAAGGGACAGUAUUCUCAG	361
AD-1143265.1	usasaagGfgAfCfAfguanucucalL96	359	asUfsgadGa(A2p)uacuguCfcCfuuuuasag	853	CUUAAAAGGGACAGUAUUCUCAG	361
AD-1143266.1	asasagGfgAfCfAfguanucucalL96	854	asUfsgadGa(Agn)uacuguCfcCfuuuuasag	855	UAAAAGGGACAGUAUUCUCAG	856
AD-1143267.1	asasagGfgAfCfAfguanucucalL96	854	asUfsgadGa(A2p)uacuguCfcCfuuuuasag	857	UAAAAGGGACAGUAUUCUCAG	856
AD-1143268.1	usasaagggdAcdAguanucucalL96	858	asUfsgadGa(Agn)uacuguCfcCfuuuuasag	852	CUUAAAAGGGACAGUAUUCUCAG	361
AD-1143269.1	asasaagggdAcdAguanucucalL96	859	asUfsgadGa(Agn)uacuguCfcCfuuuuasag	860	CUUAAAAGGGACAGUAUUCUCAG	361
AD-1143270.1	usasaagggdAcdAguanucucalL96	858	asUfsgadGa(A2p)uacuguCfcCfuuuuasag	853	CUUAAAAGGGACAGUAUUCUCAG	361
AD-1143271.1	usasaagggdAcdAguanucucalL96	861	asUfsgadGa(Agn)uacuguCfcCfuuuuasag	852	CUUAAAAGGGACAGUAUUCUCAG	361
AD-1143272.1	usasaagggdAcdAguanucucalL96	861	asUfsgadGa(A2p)uacuguCfcCfuuuuasag	853	CUUAAAAGGGACAGUAUUCUCAG	361

AD-1143273.1	usasaagggdAcdAguauucucuuL96	858	asUfsgadGadAuaucugUfcCfuuuuasag	862	CUUAAAAAGGGACAGUAUUCUCAG	361
AD-1143274.1	usasaagggdAcdAgdTuuucuuL96	861	asUfsgadGadAuaucugUfcCfuuuuasag	862	CUUAAAAAGGGACAGUAUUCUCAG	361
AD-960062.3	csusuaaaAfgGfGfAfcaguanucuuL96	377	asAfsгааUfaCfUfguccCfuUfuuaagsca	378	UGCUIAAAAAGGGACAGUAUUCUC	379
AD-1143275.1	csusuaaaAfgGfGfAfcaguanucuuL96	377	asAfsгааUfacuguccCfuUfuuaagsca	863	UGCUIAAAAAGGGACAGUAUUCUC	379
AD-1143276.1	csusuaaaAfgGfGfAfcaguanucuuL96	377	asdAsgaaUfacuguccCfuUfuuaagsca	864	UGCUIAAAAAGGGACAGUAUUCUC	379
AD-1143277.1	csusuaaaAfgGfGfAfcaguanucuuL96	377	asdAsgaaUfacuguccCfuUfuuaagscsc	865	UGCUIAAAAAGGGACAGUAUUCUC	379
AD-1143278.1	csusuaaaAfgGfGfAfcaguanucuuL96	377	asdAsgadAudAcuguccCfuUfuuaagscsc	866	UGCUIAAAAAGGGACAGUAUUCUC	379
AD-1143279.1	usasaAfgGfGfAfcaguanucuuL96	867	asdAsgaaUfacuguccCfuUfuuaasag	868	CUUAAAAAGGGACAGUAUUCUC	828
AD-1143280.1	usasaAfgGfGfAfcaguanucuuL96	867	asdAsgadAudAcuguccCfuUfuuaasag	869	CUUAAAAAGGGACAGUAUUCUC	828
AD-1143281.1	csusuaaaagdGgdAcaguanucuuL96	870	asdAsgaaUfacuguccCfuUfuuaagscsc	865	UGCUIAAAAAGGGACAGUAUUCUC	379
AD-1143282.1	csusuaaaagdGgdAcaguanucuuL96	870	asdAsgadAudAcuguccCfuUfuuaagscsc	866	UGCUIAAAAAGGGACAGUAUUCUC	379
AD-1143283.1	csusuaaaagdGgdAcdAguauucuuL96	871	asdAsgaaUfacuguccCfuUfuuaagscsc	865	UGCUIAAAAAGGGACAGUAUUCUC	379
AD-1143284.1	csusuaaaagdGgdAcdAguauucuuL96	871	asdAsgadAudAcuguccCfuUfuuaagscsc	866	UGCUIAAAAAGGGACAGUAUUCUC	379
AD-960064.3	asasaaggGfaCfaGfuaucucaguuL96	350	asCfsugaGfaAUfacugUfcCfeuuuasasa	351	UUAAAAAGGGACAGUAUUCUCAGU	352
AD-1143285.1	asasaaggGfaCfaGfuaucucaguuL96	350	asCfsugaGfaauaucugUfcCfeuuuasasa	872	UUAAAAAGGGACAGUAUUCUCAGU	352
AD-1143286.1	asasaaggGfaCfaGfuaucucaguuL96	350	asCfsugdAgdAuaucugUfcCfeuuuasasa	873	UUAAAAAGGGACAGUAUUCUCAGU	352
AD-1143287.1	asasaaggGfaCfaGfuaucucaguuL96	350	asCfsugdAgdAuaucugUfcCfeuuuasgsc	874	UUAAAAAGGGACAGUAUUCUCAGU	352
AD-1143288.1	asasaaggGfaCfaGfuaucucaguuL96	350	asCfsugdAgdAuaucugUfcCfeuuuasgsc	875	UUAAAAAGGGACAGUAUUCUCAGU	352
AD-1143289.1	asasaaggGfaCfaGfuaucucaguuL96	876	asCfsugdAgdAuaucugUfcCfeuuusuu	877	AAAAAGGGACAGUAUUCUCAGU	878

AD-1143290.1	asasaagggaCfadGuauncucagauL96	879	asCfsugdAgdAuaucgUfcCfeuuusgsc	874	UUAAAAGGGACAGUAUUCUCAGU	352
AD-1143291.1	asasaagggaCfadGuauncucagauL96	879	asCfsugdAgdAuaucgUfcCfeuuusesc	875	UUAAAAGGGACAGUAUUCUCAGU	352
AD-1143292.1	asasaagggaCfadGuauncucagauL96	880	asCfsugdAadAuaucgUfcCfeuuusgsc	881	UUAAAAGGGACAGUAUUCUCAGU	352
AD-1143293.1	asasaagggaCfadGuauncucagauL96	882	asCfsugdAgdAuaucgUfcCfeuuusgsc	874	UUAAAAGGGACAGUAUUCUCAGU	352
AD-1143294.1	asasaagggaCfadGuauncucagauL96	882	asCfsugdAgdAuaucgUfcCfeuuusesc	875	UUAAAAGGGACAGUAUUCUCAGU	352
AD-1143295.1	asasaagggaCfadGuauncucagauL96	883	asCfsugdAgdAuaucgUfcCfeuuusgsc	874	UUAAAAGGGACAGUAUUCUCAGU	352
AD-1143296.1	asasaagggaCfadGuauncucagauL96	883	asCfsugdAgdAuaucgUfcCfeuuusesc	875	UUAAAAGGGACAGUAUUCUCAGU	352
AD-960096.3	cscsaauaAfaGfCfUfggacaagaauL96	347	asUfsueuUfgUfcfcageUfuUfaungsgsgsa	348	UCCCAAUAAAAGCUGGACAAAGAAAG	349
AD-1143297.1	cscsaauaAfaGfCfUfggacaagaauL96	884	asUfsueuUfaucageUfuUfaungsgsgsa	885	UCCCAAUAAAAGCUGGACAAAGAAAG	349
AD-1143298.1	cscsaauaAfaGfCfUfggacaagaauL96	347	asUfsueuTu(G2p)uccageUfuUfaungsgsgsa	886	UCCCAAUAAAAGCUGGACAAAGAAAG	349
AD-1143299.1	cscsaauaAfaGfCfUfggacaagaauL96	347	asUfsueuTu(G2p)uccageUfuUfaungsgsgsc	887	UCCCAAUAAAAGCUGGACAAAGAAAG	349
AD-1143300.1	asasuaAfaGfCfUfggacaagaauL96	888	asUfsueuTu(G2p)uccageUfuUfaungsgsg	889	CCAAUAAAAGCUGGACAAAGAAAG	890
AD-1143301.1	asasuaAfaGfCfUfggacaagaauL96	888	asUfsueuTu(G2p)uccageUfuUfaungsgsc	891	CCAAUAAAAGCUGGACAAAGAAAG	890
AD-1143302.1	cscsaauaAfaGfCfUfggacaagaauL96	892	asUfsueuTu(G2p)uccageUfuUfaungsgsgsc	887	UCCCAAUAAAAGCUGGACAAAGAAAG	349
AD-1143303.1	cscsaauaAfaGfCfUfggacaagaauL96	892	asUfsueuTu(G2p)uccageUfuUfaungsgsg	889	CCAAUAAAAGCUGGACAAAGAAAG	890
AD-1143304.1	cscsaauadAagCfUfggacaagaauL96	893	asUfsueuTu(G2p)uccageUfuUfaungsgsgsc	887	UCCCAAUAAAAGCUGGACAAAGAAAG	349
AD-1143305.1	cscsaauadAagCfUfggacaagaauL96	893	asUfsueuTu(G2p)uccageUfuUfaungsgsg	889	CCAAUAAAAGCUGGACAAAGAAAG	890
AD-1143306.1	cscsaauaAfaGfCfUfggacaagaauL96	892	asUfsueuUfgucageUfuUfaungsgsgsa	894	UCCCAAUAAAAGCUGGACAAAGAAAG	349
AD-1143307.1	cscsaauadAagCfUfggacaagaauL96	893	asUfsueuUfgucageUfuUfaungsgsgsa	894	UCCCAAUAAAAGCUGGACAAAGAAAG	349

AD-960481.3	asagcugGfaCfaAfAfgaagcugcuuL96	454	asAfsgeag(Cgn)uucuuUfcCfagcuususa	718	UAAAGCUGGACAAAGAAAGCUGCUA	456
AD-1143308.1	asagcugGfaCfaAfAfgaagcugcuuL96	454	asAfsgeadAg(Cgn)uucuuUfcCfagcuususa	895	UAAAGCUGGACAAAGAAAGCUGCUA	456
AD-1143309.1	asagcugGfaCfaAfAfgaagcugcuuL96	454	asdAfsgeadAg(Cgn)uucuuUfcCfagcuususa	896	UAAAGCUGGACAAAGAAAGCUGCUA	456
AD-1143310.1	asagcugGfaCfaAfAfgaagcugcuuL96	454	asdAfsgeadAg(C2p)uucuuUfcCfagcuususa	897	UAAAGCUGGACAAAGAAAGCUGCUA	456
AD-1143311.1	asagcugGfaCfaAfAfgaagcugcuuL96	454	asdAfsgeadAg(Cgn)uucuuUfcCfagcuususc	898	UAAAGCUGGACAAAGAAAGCUGCUA	456
AD-1143312.1	asagcugGfaCfaAfAfgaagcugcuuL96	454	asdAfsgeadAg(C2p)uucuuUfcCfagcuususc	899	UAAAGCUGGACAAAGAAAGCUGCUA	456
AD-1143313.1	asagcugGfaCfaAfAfgaagcugcuuL96	454	asdAfsgeadAg(Cgn)uucuuUfcCfagcuususc	900	UAAAGCUGGACAAAGAAAGCUGCUA	456
AD-1143314.1	asagcugGfaCfaAfAfgaagcugcuuL96	454	asdAfsgeadAg(C2p)uucuuUfcCfagcuususc	901	UAAAGCUGGACAAAGAAAGCUGCUA	456
AD-1143315.1	gscsugGfaCfaAfAfgaagcugcuuL96	902	asdAfsgeadAg(Cgn)uucuuUfcCfagcsusu	903	AAGCUGGACAAAGAAAGCUGCUA	904
AD-1143316.1	gscsugGfaCfaAfAfgaagcugcuuL96	902	asdAfsgeadAg(C2p)uucuuUfcCfagcsusu	905	AAGCUGGACAAAGAAAGCUGCUA	904
AD-1143317.1	asagcugGfaCfaAfAfgaagcugcuuL96	906	asdAfsgeadAg(Cgn)uucuuUfcCfagcuususc	900	UAAAGCUGGACAAAGAAAGCUGCUA	456
AD-1143318.1	asagcugGfaCfaAfAfgaagcugcuuL96	906	asdAfsgeadAg(C2p)uucuuUfcCfagcuususc	901	UAAAGCUGGACAAAGAAAGCUGCUA	456
AD-1143319.1	asagcugGfaCfaAfAfgaagcugcuuL96	907	asdAfsgeadAg(Cgn)uucuuUfcCfagcuususc	900	UAAAGCUGGACAAAGAAAGCUGCUA	456
AD-1143320.1	asagcugGfaCfaAfAfgaagcugcuuL96	907	asdAfsgeadAg(C2p)uucuuUfcCfagcuususc	901	UAAAGCUGGACAAAGAAAGCUGCUA	456
AD-1143321.1	asagcugGfaCfaAfAfgaagcugcuuL96	908	asdAfsgeadAg(C2p)uucuuUfcCfagcuususc	909	UAAAGCUGGACAAAGAAAGCUGCUA	456
AD-1143322.1	asagcugGfaCfaAfAfgaagcugcuuL96	910	asdAfsgeadAg(C2p)uucuuUfcCfagcuususc	911	UAAAGCUGGACAAAGAAAGCUGCUA	456
AD-1183925	asagcugGfaCfaAfAfgaagcugcuuL96	912	asdAfsgeadAg(C2p)uucuuUfcCfagcuususc	899	UAAAGCUGGACAAAGAAAGCUGCUA	456
AD-1183926	gscsugGfaCfaAfAfgaagcugcuuL96	913	asdAfsgeadAg(C2p)uucuuUfcCfagcsusu	905	AAGCUGGACAAAGAAAGCUGCUA	904
AD-1019001	Y44sacgggacaGfUfAfAfuucucaguiasY44	914	usCfsasCfuGfagaauAfcUfgUfcCfagcsusu	915	AAGGACAGUAUUCUCAGUGC	916

Table 16. Single Dose Free Uptake Screens in Hep3B cells

Duplex Name	500nM Avg	SD	100nM Avg	SD	10nM Avg	SD	1nM Avg	SD
AD-80794.10	50.01	2.91	57.23	3.03	73.67	4.07	92.13	2.45
AD-1143240.1	59.83	5.91	62.99	4.12	75.71	3.36	90.44	4.01
AD-1143241.1	46.00	2.20	51.40	2.74	70.00	5.07	87.46	2.15
AD-1143242.1	46.98	2.67	57.58	4.13	74.48	4.89	85.07	2.08
AD-1143243.1	32.17	2.39	37.22	2.97	62.41	5.86	83.22	3.83
AD-1143244.1	61.26	3.79	65.51	3.33	70.95	4.64	86.55	1.88
AD-1143245.1	29.99	2.62	38.17	2.32	57.74	5.64	81.92	3.13
AD-1143246.1	104.48	22.29	76.68	3.36	84.88	3.76	91.31	6.20
AD-1143247.1	49.96	1.22	57.75	3.51	76.50	6.15	86.40	1.72
AD-1143248.1	76.38	2.07	81.24	4.83	90.92	6.41	91.34	2.56
AD-1143249.1	47.94	1.92	53.80	3.52	74.14	2.76	89.48	3.45
AD-960030.3	41.64	0.96	49.21	2.30	70.81	4.30	90.77	7.86
AD-1143250.1	43.66	1.99	50.06	2.06	71.09	1.41	88.52	1.54
AD-1143251.1	44.46	2.85	53.43	0.48	70.58	4.73	87.93	2.59
AD-1143252.1	35.28	4.47	39.27	3.04	63.45	2.88	84.67	3.62
AD-1143253.1	45.97	4.59	48.91	3.12	72.50	3.30	85.82	2.60
AD-1143254.1	33.14	1.27	39.94	2.86	67.59	4.55	85.51	1.63
AD-1143255.1	22.44	3.31	33.91	6.67	53.15	3.97	80.09	3.20
AD-1143256.1	12.09	1.29	18.11	1.50	54.06	9.34	75.50	2.77
AD-1143257.1	108.85	5.61	93.98	5.84	92.13	5.41	93.92	3.11
AD-1143258.1	84.74	1.55	82.30	2.97	85.54	5.46	93.59	2.95
AD-1143259.1	84.03	3.05	88.74	1.41	85.29	2.51	100.73	15.35
AD-1143260.1	87.46	3.43	78.59	4.36	83.55	2.88	91.04	3.38
AD-960031.3	29.38	3.85	41.40	6.94	62.15	5.10	83.65	3.72
AD-1143261.1	96.26	12.69	97.98	11.29	88.10	5.65	94.22	4.29
AD-1143262.1	76.29	3.64	75.61	2.89	90.82	7.38	95.42	4.11
AD-1143263.1	48.38	9.73	61.04	5.42	81.86	3.97	92.05	5.07
AD-1143264.1	66.67	3.82	76.51	1.92	84.98	1.39	91.16	1.37
AD-1143265.1	68.17	13.37	77.06	12.29	81.83	3.04	92.15	6.59
AD-1143266.1	74.35	11.32	68.43	4.30	82.16	4.30	96.14	13.04
AD-1143267.1	44.99	5.20	64.81	10.27	78.18	9.28	89.68	4.66
AD-1143268.1	75.66	8.75	91.75	14.39	89.63	5.81	100.77	5.48
AD-1143269.1	70.81	3.28	84.12	5.28	95.03	5.00	97.24	7.30
AD-1143270.1	71.74	4.79	78.83	3.42	92.89	4.17	95.82	5.96
AD-1143271.1	71.77	5.30	76.02	3.43	91.08	9.11	95.56	3.23
AD-1143272.1	69.18	1.04	77.97	1.16	91.30	7.61	90.72	2.88
AD-1143273.1	46.11	5.48	58.47	5.63	79.00	5.34	86.46	2.44
AD-1143274.1	57.35	7.81	59.49	3.88	82.43	4.08	94.65	5.38
AD-960062.3	42.21	6.42	58.97	13.39	76.38	11.22	100.56	15.42
AD-1143275.1	54.25	10.60	67.40	11.53	80.59	6.62	108.48	13.17
AD-1143276.1	57.96	3.63	64.19	4.11	80.40	1.72	91.66	2.79
AD-1143277.1	60.46	6.29	73.08	8.35	114.87	n/a	95.41	7.44

Duplex Name	500nM Avg	SD	100nM Avg	SD	10nM Avg	SD	1nM Avg	SD
AD-1143278.1	30.74	2.87	46.93	7.31	75.59	3.54	90.06	12.78
AD-1143279.1	46.16	2.13	63.91	11.43	84.18	4.57	89.02	3.39
AD-1143280.1	26.65	6.19	39.95	8.20	71.49	2.71	84.48	2.10
AD-1143281.1	83.25	5.26	97.82	9.56	87.57	5.49	95.78	5.34
AD-1143282.1	62.39	5.01	70.93	12.74	77.38	0.93	92.25	3.23
AD-1143283.1	74.72	3.35	81.27	5.49	92.84	2.43	100.14	3.42
AD-1143284.1	53.26	1.19	63.20	4.02	83.55	3.21	95.11	4.68
AD-960064.3	35.24	4.68	45.96	3.22	73.26	3.33	88.25	3.26
AD-1143285.1	26.79	2.81	34.47	4.00	77.33	6.80	86.04	9.70
AD-1143286.1	19.35	3.41	29.77	4.83	67.58	14.28	88.54	12.85
AD-1143287.1	36.62	2.41	40.55	10.58	64.21	2.67	88.54	12.76
AD-1143288.1	45.07	9.60	50.93	11.36	74.56	9.65	87.44	7.30
AD-1143289.1	18.55	4.13	26.32	5.27	59.92	6.61	85.97	12.38
AD-1143290.1	66.75	2.91	75.76	4.76	89.92	10.03	103.24	6.45
AD-1143291.1	61.61	1.41	74.92	7.37	96.58	6.23	92.67	4.65
AD-1143292.1	103.71	13.87	96.00	6.82	102.08	18.11	89.10	2.78
AD-1143293.1	64.75	7.70	70.52	5.16	82.76	2.30	88.53	6.30
AD-1143294.1	66.74	10.80	69.22	11.38	80.55	4.13	100.67	14.77
AD-1143295.1	62.66	9.65	62.74	10.64	89.92	20.11	99.75	17.24
AD-1143296.1	68.47	8.32	74.41	5.35	89.72	15.96	102.45	15.29
AD-960096.3	62.06	9.14	62.82	3.97	73.10	3.78	98.71	13.86
AD-1143297.1	63.46	2.13	64.77	0.99	94.66	15.45	87.45	1.11
AD-1143298.1	36.30	4.11	45.39	2.87	77.72	6.66	88.99	6.44
AD-1143299.1	63.33	7.70	60.86	4.26	84.28	16.31	83.82	4.27
AD-1143300.1	31.00	5.52	36.78	5.31	75.23	10.17	89.41	15.09
AD-1143301.1	63.14	13.06	51.90	3.83	83.74	24.11	97.24	14.28
AD-1143302.1	65.92	4.89	72.42	3.43	82.76	5.03	94.16	5.49
AD-1143303.1	72.20	8.33	70.63	2.96	89.97	10.86	101.84	15.93
AD-1143304.1	55.09	2.79	63.03	4.77	78.29	1.36	82.37	3.89
AD-1143305.1	56.12	9.04	58.35	3.55	85.76	6.85	95.38	7.01
AD-1143306.1	95.15	16.35	87.12	1.78	94.75	12.83	93.02	5.19
AD-1143307.1	101.10	14.18	89.16	9.67	98.12	17.65	97.60	10.55
AD-960481.3	76.27	13.63	63.55	6.29	94.32	27.33	96.57	14.20
AD-1143308.1	53.08	3.84	60.34	1.74	73.24	2.55	89.76	1.87
AD-1143309.1	65.24	6.58	68.94	9.69	87.87	12.46	89.04	4.03
AD-1143310.1	60.98	1.83	65.14	2.41	80.93	11.19	86.62	7.35
AD-1143311.1	61.07	0.91	61.76	3.10	91.14	13.28	87.45	1.73
AD-1143312.1	61.85	14.18	53.82	1.28	85.58	8.53	83.02	5.64
AD-1143313.1	79.09	10.91	68.96	8.44	114.58	9.72	84.57	5.14
AD-1143314.1	65.98	14.04	54.39	4.13	97.29	9.49	85.95	15.06
AD-1143315.1	69.73	9.31	56.52	4.43	89.23	19.66	87.97	11.47
AD-1143316.1	37.58	9.74	45.48	4.36	67.15	13.63	86.39	8.46
AD-1143317.1	75.31	5.07	77.89	3.77	79.47	3.23	89.32	2.95
AD-1143318.1	88.04	2.18	85.20	2.80	84.34	4.84	92.15	3.21

Duplex Name	500nM Avg	SD	100nM Avg	SD	10nM Avg	SD	1nM Avg	SD
AD-1143319.1	78.11	1.03	78.66	1.90	85.48	3.98	87.83	4.39
AD-1143320.1	72.96	4.16	76.75	5.12	83.72	3.86	86.10	1.86
AD-1143321.1	88.56	4.22	87.05	4.63	92.98	16.13	86.86	2.20
AD-1143322.1	96.56	8.01	87.69	5.49	94.48	14.95	90.38	1.88

Table 17. APOC3 Single Dose Screens in Hep3B cells

Duplex Name	50nM Avg	SD	10nM Avg	SD	1nM Avg	SD	0.1nM Avg	SD
AD-80794.10	5.7	1.1	9.95	0.48	32.69	7.14	64.32	3.18
AD-1143240.1	7.1	1.2	15.92	1.88	38.52	6.25	69.61	5.09
AD-1143241.1	5.7	0.4	10.24	0.34	25.95	2.96	57.20	3.42
AD-1143242.1	6.4	0.9	13.22	1.13	34.61	3.94	61.90	6.43
AD-1143243.1	3.6	1.2	4.93	1.44	11.97	2.19	41.16	3.87
AD-1143244.1	6.1	0.6	14.76	2.30	35.03	5.30	63.06	1.15
AD-1143245.1	3.2	1.0	4.79	0.38	13.05	3.50	35.33	4.64
AD-1143246.1	23.5	2.7	48.43	3.38	51.77	11.43	62.50	8.17
AD-1143247.1	8.2	1.5	15.58	1.25	33.36	3.62	67.35	1.28
AD-1143248.1	30.0	7.8	42.16	4.87	71.78	8.67	90.87	8.70
AD-1143249.1	6.7	1.3	10.12	1.10	30.61	2.16	73.82	12.68
AD-960030.3	6.3	1.2	10.86	0.88	33.81	1.58	78.29	7.19
AD-1143250.1	7.3	2.6	11.13	3.32	38.58	5.25	74.13	15.33
AD-1143251.1	5.7	0.4	11.28	2.82	26.55	4.13	73.77	9.73
AD-1143252.1	3.6	0.8	7.27	1.30	14.89	2.35	60.85	12.26
AD-1143253.1	3.7	0.9	9.57	2.15	24.55	4.12	66.79	6.79
AD-1143254.1	3.4	0.7	5.32	2.07	14.73	2.96	53.26	5.75
AD-1143255.1	4.6	1.3	5.67	1.06	21.00	4.11	71.94	13.73
AD-1143256.1	3.3	0.6	4.51	0.83	11.84	1.22	44.47	5.74
AD-1143257.1	42.1	5.6	66.28	4.64	77.50	16.10	85.21	5.60
AD-1143258.1	14.8	2.8	30.73	2.64	50.53	2.24	76.78	4.31
AD-1143259.1	39.3	10.8	62.34	10.95	58.31	9.70	92.23	23.63
AD-1143260.1	10.3	4.1	27.71	4.89	46.07	6.70	76.35	6.59
AD-960031.3	4.1	0.5	6.33	1.49	13.71	3.98	52.14	2.03
AD-1143261.1	30.0	7.0	45.40	7.52	76.11	11.73	96.35	13.51
AD-1143262.1	8.7	0.6	12.67	2.42	33.93	4.28	80.72	10.76
AD-1143263.1	7.0	1.9	8.59	1.53	24.97	4.76	70.04	3.98
AD-1143264.1	10.5	2.6	15.57	2.68	36.20	4.20	69.58	4.36
AD-1143265.1	7.0	1.1	14.90	4.74	29.19	7.67	68.07	6.60
AD-1143266.1	7.4	3.6	14.06	3.79	36.54	10.79	69.23	16.16
AD-1143267.1	6.4	2.8	10.39	4.74	27.85	9.35	77.07	14.58
AD-1143268.1	18.4	5.3	30.21	7.20	64.69	15.99	87.24	6.39
AD-1143269.1	12.7	1.0	23.02	5.11	48.27	3.18	89.49	12.32
AD-1143270.1	11.9	1.8	23.75	6.38	48.58	8.61	85.30	6.72
AD-1143271.1	15.6	2.4	22.50	4.14	51.50	15.12	75.61	4.59

Duplex Name	50nM Avg	SD	10nM Avg	SD	1nM Avg	SD	0.1nM Avg	SD
AD-1143272.1	13.6	0.7	23.31	5.30	48.00	6.30	79.88	3.87
AD-1143273.1	6.5	1.7	10.62	2.32	30.43	12.95	59.42	3.85
AD-1143274.1	5.9	1.9	9.74	2.72	20.44	5.25	59.55	8.94
AD-960062.3	6.0	1.1	14.90	3.08	38.29	5.48	78.23	11.31
AD-1143275.1	21.9	20.0	20.20	4.71	58.80	19.25	87.53	3.36
AD-1143276.1	13.5	4.1	17.22	1.37	45.40	5.79	81.50	8.12
AD-1143277.1	11.2	2.2	17.51	2.37	42.03	6.65	81.04	9.28
AD-1143278.1	5.0	1.4	6.79	0.82	15.99	2.77	58.30	14.40
AD-1143279.1	7.6	2.0	11.26	1.68	32.37	4.56	62.14	3.43
AD-1143280.1	4.8	3.0	5.89	0.52	13.05	3.98	41.79	5.31
AD-1143281.1	45.7	5.9	59.48	7.68	70.38	3.75	77.63	7.52
AD-1143282.1	16.3	2.4	24.29	6.42	56.76	5.55	80.93	3.65
AD-1143283.1	25.8	1.5	44.82	11.38	72.55	14.90	94.36	5.31
AD-1143284.1	10.4	2.8	16.97	2.34	42.91	5.08	79.91	4.59
AD-960064.3	4.2	0.6	7.37	3.78	19.17	4.36	49.10	5.66
AD-1143285.1	5.4	1.4	7.86	1.22	17.89	1.37	54.59	14.15
AD-1143286.1	4.0	1.4	5.02	1.00	14.71	4.90	39.71	1.47
AD-1143287.1	4.7	1.9	5.59	2.40	15.79	4.39	48.62	13.52
AD-1143288.1	6.7	1.6	6.50	0.81	21.80	8.36	64.64	18.78
AD-1143289.1	4.8	0.7	7.27	1.89	19.96	2.45	72.86	11.19
AD-1143290.1	13.1	3.0	21.10	3.85	56.96	2.68	84.38	17.46
AD-1143291.1	11.3	0.7	20.46	3.83	57.48	7.36	83.09	13.09
AD-1143292.1	73.7	6.0	76.74	12.40	85.93	13.10	85.38	4.81
AD-1143293.1	9.3	1.3	20.27	4.53	42.95	5.74	73.75	2.06
AD-1143294.1	12.1	1.4	25.36	7.70	49.62	7.18	86.00	5.63
AD-1143295.1	8.3	2.3	12.41	2.06	36.35	1.40	58.61	9.02
AD-1143296.1	9.8	1.2	18.77	6.23	47.68	14.59	85.91	4.26
AD-960096.3	8.5	1.9	10.64	2.21	28.32	4.33	69.12	1.44
AD-1143297.1	8.7	0.7	14.90	2.16	51.05	16.48	90.58	15.04
AD-1143298.1	4.9	0.3	6.62	0.97	18.55	4.52	65.95	17.11
AD-1143299.1	5.5	1.2	7.73	1.65	22.91	1.45	59.57	8.18
AD-1143300.1	4.9	1.2	8.31	3.19	22.19	11.16	52.43	7.83
AD-1143301.1	6.3	0.9	10.75	1.98	28.49	7.31	70.30	10.58
AD-1143302.1	7.9	1.5	11.19	3.20	38.83	14.98	87.33	15.21
AD-1143303.1	8.6	1.3	12.40	2.65	48.46	1.79	98.13	17.19
AD-1143304.1	9.7	2.5	11.07	1.79	35.65	3.16	93.11	17.36
AD-1143305.1	9.1	4.3	10.51	2.17	36.75	4.73	75.09	5.82
AD-1143306.1	41.1	6.7	58.50	7.88	85.88	16.83	85.51	2.76
AD-1143307.1	37.6	4.0	48.21	1.96	90.07	19.23	99.33	19.17
AD-960481.3	14.3	3.0	16.70	1.93	24.93	4.18	58.59	12.39
AD-1143308.1	10.4	0.9	11.43	0.75	20.45	6.28	55.52	9.39
AD-1143309.1	15.5	1.4	16.40	2.68	34.69	2.64	67.90	12.70
AD-1143310.1	13.8	1.0	15.28	1.15	30.24	5.65	68.82	8.91
AD-1143311.1	15.4	2.4	17.79	2.39	34.59	7.22	65.86	10.52

Duplex Name	50nM Avg	SD	10nM Avg	SD	1nM Avg	SD	0.1nM Avg	SD
AD-1143312.1	14.5	1.6	15.72	3.57	31.73	11.40	57.08	6.92
AD-1143313.1	20.8	3.4	27.95	14.33	42.27	7.42	75.75	8.10
AD-1143314.1	17.1	2.1	19.52	2.15	37.72	8.99	72.95	22.77
AD-1143315.1	12.7	3.7	17.40	3.53	31.01	8.81	76.52	15.22
AD-1143316.1	11.8	0.8	14.83	1.86	30.60	5.14	67.90	8.60
AD-1143317.1	18.8	2.0	22.92	3.28	44.93	5.70	70.55	6.43
AD-1143318.1	35.6	2.2	34.82	2.89	59.98	8.35	75.30	3.45
AD-1143319.1	15.9	1.3	21.27	3.79	41.72	6.38	70.74	12.44
AD-1143320.1	23.8	1.4	33.05	5.65	59.79	7.71	68.64	11.31
AD-1143321.1	52.4	6.7	60.14	7.06	73.20	5.57	71.89	1.25
AD-1143322.1	51.0	3.8	62.29	7.70	67.07	7.38	78.84	15.84

Duplexes of interest, identified from the above *in vitro* SAR studies, were evaluated *in vivo*.

- In particular, at pre-dose day -14 wild-type mice (C57BL/6) were transduced by retroorbital administration of 2×10^{10} viral particles of an adeno-associated virus 8 (AAV8) vector encoding human APOC3. In particular, mice were administered an AAV8 encoding the human APOC3 mRNA, referred to as AAV8-TBG-PI-APOC3.

- At day 0, groups of three mice were subcutaneously administered a single 3 mg/kg dose of the agents of interest or PBS control. Table 18 provides the treatment groups and Table 19 provides the duplexes of interest. At day 7 or day 14 post-dose animals were sacrificed, liver samples were collected and snap-frozen in liquid nitrogen. Liver mRNA was extracted and analyzed by the RT-QPCR method.

- Human APOC3 mRNA levels were compared to a housekeeping gene, GAPDH. The values were then normalized to the average of PBS vehicle control group. The data were expressed as percent of baseline value, and presented as mean plus standard deviation. The results, listed in Table 20 and shown in Figure 3, demonstrate that the exemplary duplex agents tested effectively reduce the level of the human APOC3 messenger RNA *in vivo*.

Table 18. Treatment Groups

Group #	Animal #	Treatment	Dose	Timepoint
1	1	PBS	n/a	D0, D7, D14
	2			
	3			
2	4	Naïve (AAV only)	n/a	
	5			
	6			
3	7	AD-80794 (Benchmark)	3mpk	
	8			
	9			

4	10	AD-960030
	11	
	12	
5	13	AD-1143243
	14	
	15	
6	16	AD-1143245
	17	
	18	
7	19	AD-1143247
	20	
	21	
8	22	AD-1143249
	23	
	24	
9	25	AD-1143256
	26	
	27	
10	28	AD-1143289
	29	
	30	
11	31	AD-1143278
	32	
	33	
12	34	AD-1143287
	35	
	36	
13	37	AD-1143295
	38	
	39	
14	40	AD-1143299
	41	
	42	
15	43	AD-1143302
	44	
	45	
16	46	AD-1143305
	47	
	48	
17	49	AD-1183925
	50	
	51	
18	52	AD-

	53	1183926	
	54		
19	55	AD-1019001 (Arrowhead)	
	56		
	57		
20	58	AD-58295	
	59		
	60		

Table 19. Duplexes of Interest

DuplexID	Range in NM-000040.3
AD-960030	429-451
AD-1143243	432-452
AD-1143245	432-452
AD-1143247	430-452
AD-1143249	430-452
AD-1143256	431-451
AD-1143260	429-451
AD-1143278	430-452
AD-1143287	433-455
AD-1143295	433-455
AD-1143299	504-526
AD-1143302	504-526
AD-1143305	506-526
AD-1183925	510-532
AD-1183926	512-532
AD-58295	Negative Control

Table 20.

Duplex	% Message Remaining	SD
PBS	102.64	27.23
AD-80794.11	19.86	9.05
AD-960030.4	16.99	4.69
AD-1143243.2	25.98	5.12
AD-1143245.2	39.13	14.55
AD-1143247.2	41.77	56.07
AD-1143249.2	31.91	32.10
AD-1143256.2	15.43	7.40
AD-1143289.2	8.27	1.73
AD-1143278.2	54.31	12.95
AD-1143287.2	4.00	4.94
AD-1143295.2	24.85	18.86

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AD-1143299.2	23.37	5.80
AD-1143302.2	57.80	27.34
AD-1143305.2	38.33	23.55
AD-1183925.2	31.91	5.66
AD-1183926.2	22.87	6.44
AD-1019001.2	99.93	32.38
AD-58295.5	84.59	43.87

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments and methods described herein. Such equivalents are intended to be encompassed by the scope of the following claims.

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